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AIM AND SCOPE

Viral Hepatitis Journal (Formerly Viral Hepatit Dergisi) is the regular publishing organ of the Viral Hepatitis Society. This periodical journal covers diagnosis, treatment, epidemiology, prevention and information of hepatitis.

Viral Hepatitis Journal is an open-access journal published 3 times per year (April, August and December). In addition, the special issues are published in some periods. It is a periodic national/international journal, published in English language with abstract and title published also in Turkish language and its editorial policies are based on independent peer-review principles.

The aim of Viral Hepatitis Journal is to continuously publish original research papers of the highest scientific and clinical values specifically on hepatitis, on an international level. Additionally, reviews on basic developments in education, editorial short notes, case reports, original views, letters from a wide range of medical personal containing experiences and comments as well as social subjects are published.

For general practitioners giving first line medical service who are interested in hepatitis, specialists in internal medicine, gastroenterology, microbiology, family physician, public health and hepatology, 'things that must be known' subjects will ensure to involve in Viral Hepatitis Journal.

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Viral Hepatitis Journal is a scientific journal that publishes retrospective, prospective or experimental research articles, review articles, case reports, editorial comment/discussion, letter to the editor, surgical technique, differential diagnosis, medical book reviews, questions-answers and also current issues of medical agenda from all fields of medicine and aims to reach all national/international institutions and individuals.

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The author(s) undertake(s) all scientific responsibility for the manuscript. All the authors must actively participate in the study. The author(s) guarantee(s) that the manuscript itself or any substantially similar content of the manuscript has not been published or is being considered for publication elsewhere. If the manuscript had been presented in a meeting before; the name, date and the province of the meeting should be noted.

Experimental, clinical and drug studies requiring approval by an ethics committee must be submitted to the Viral Hepatitis Journal with an ethics committee approval report confirming that the study was conducted in accordance with international agreements and the Declaration of Helsinki (revised in 2013) (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The approval of the ethics committee and the fact that informed consent was given by the patients should be indicated in the Materials and Methods section (including approval number). All papers reporting experiments using animals must include a statement in the Material and Methods section giving assurance that all animals have received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html) and indicating approval by the institutional ethical review board.

The content of the submitted manuscripts should conform to the criteria stated in "Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals" published by International Committee of Medical Journal Editors and updated in 2016 (available at http://www.icmje.org/).

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The name of the institution where the authors work and the name of the institution or the department in which the study has been conducted should not be mentioned in the submitted manuscript.

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The result can be acceptance, minor revision, major revision, rejection in the current form, or rejection. Accepted manuscripts are forwarded for publication; in this stage, all information and data are checked and controlled properly; the proof of the article to be published by the journal are forwarded to the writers for proof reading and corrections.

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Preparation of research articles and systematic reviews meta-analyses must comply with study design guidelines: CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/),

PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

STROBE statement—checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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Authors are encouraged to follow the following principles before submitting their article:

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Short Announcements

i. Turkish title, English title, author(s)' name(s) and institution(s) (Turkish and English)
ii. Turkish and English Abstract (max 300 words)
iii. Turkish and English Keywords
iv. Introduction (max 300 words)
v. Materials and Methods (max 400 words)
vi. Results (max 400 words)
vii. Discussion (max 700 words)
viii. Referances (should not exceed 15), all words 2000 not exceed.

• Author number for review articles should not exceed three.

• Author number for case report presentations should not exceed four.

• Articles should be written with double line space in 10 font size and right, left, upper and lower margins should all be 2.5 cm. Writing style should be Arial.

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Manuscripts should be written with Microsoft Word and the main text should not exceed 2000 words.

Abbreviations: Abbreviations should be defined at first mention and used consistently thereafter. Internationally accepted abbreviations should be used; refer to scientific writing guides as necessary.

Cover Letter: Cover letter should include statements about manuscript category designation, single-journal submission affirmation, conflict of interest statement, sources of outside funding, equipments (if so), approval for language for articles in English and approval for statistical analysis for original research articles.

Title Page: Title should be concise and informative (in Turkish and English). The title page should include a list of all contributing authors and all of their affiliations. Positions of authors and names of departments and institutions to which they are attached and the province should be written. Supply full correspondence details for the corresponding author, including phone, mobile phone, fax number and e-mail address.

ARTICLE SECTIONS

The text file should include the title in Turkish, keywords, the title in English, keywords in English, the text of the article, references, tables (only one table for one page) and figure

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legends (if any), respectively. Within the text file, the names of the authors, any information about the institutions, the figures and images should be excluded.

Abstract: Turkish and English abstracts should be given together with the article title. It should be divided into four sections in the following order: Objectives, Materials and Methods, Results and Conclusion. Abstracts should not exceed 250 words. Abstracts for case reports should be unstructured and shorter (average 100-150 words; without structural divisions in Turkish and English).

Objectives: The aim of the study should be clearly stated.

Materials and Methods: **The study and standard criteria used should be defined; it should also** be indicated whether the study is randomized or not, whether it is retrospective or prospective, and the statistical methods applied should be indicated, if applicable.

Results: The detailed results of the study should be given and the statistical significance level should be indicated.

Conclusion: Should summarize the results of the study, the clinical applicability of the results should be defined, and the favorable and unfavorable aspects should be declared.

Keywords:

• They should be minimally 3 and maximally 6 and should be written in Turkish and English.

• The words should be separated by semicolon (;) from each other.

• English keywords should be appropriate to "Medical Subject Headings (MESH)" (www.nlm.nih.gov/mesh/MBrowser.html).

• Turkish keywords should be appropriate to "Turkey Science Terms" (www.bilimterimleri.com).

Original researches should have the following sections;

Introduction: Should consist of a brief explanation of the topic and indicate the objective of the study, supported by information from the literature.

Materials and Methods: The study plan should be clearly described, indicating whether the study is randomized or not, whether it is retrospective or prospective, the number of trials, the characteristics, and the statistical methods used.

Results: The results of the study should be stated, with tables/figures given in numerical order; the results should be evaluated according to the statistical analysis methods applied. See General Guidelines for details about the preparation of visual material.

Discussion: The study results should be discussed in terms of their favorable and unfavorable aspects and they should be compared with the literature.

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Conclusion: The conclusion of the study should be highlighted.

Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English/Turkish evaluation) towards the study should appear at the end of the article. Only acknowledge persons and institutions who have made substantial contributions to the study, but was not a writer of the paper.

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Case Reports

Case reports should present cases which are rarely seen, feature novelty in diagnosis and treatment, and contribute to our current knowledge. The first page should include the title in Turkish and English, an unstructured summary not exceeding 150 words, and keywords. The main text should consist of introduction, case report, discussion, acknowledgment, conclusion and references. The entire text should not exceed 5 pages (A4, formatted as specified above).

Review Articles

Review articles can address any aspect of viral hepatitis Review articles must provide critical analyses of contemporary evidence and provide directions of or future research. Most review articles are commissioned, but other review submissions are also welcome. Before sending a review, discussion with the editor is recommended.

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Letters to the Editor should be short commentaries related to current developments in viral hepatitis and their scientific and social aspects, or may be submitted to ask questions or offer further contributions in response to work that has been published in the Viral Hepatitis Journal. Letters do not include a title or an abstract; they should not exceed 1000 words and can have up to 5 references.

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Follow the styles shown in examples below (please give attention to punctuation):

In reference section of the article, there should be no writing in languages other than English. The text language of the article should be indicated in parenthesis at the end of each reference (e.g. Yoldaş O, Bulut A, Altındiş M. The Current Approach of Hepatitis A Infections. Viral Hepatitis J 2012;18:81-86. (Turkish).

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Example: Tabak F, Ozdemir F, Tabak O, Erer B, Tahan V, Ozaras R. Autoimmune hepatitis induced by the prolonged hepatitis A virus infection. Ann Hepatol. 2008;7:177-179.

Format for books; initials of author's names and surnames, chapter title, editor's name, book title, edition, city, publisher, date and pages.

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• Title Page

• Article sections

• Turkish and English titles

• Abstract (250 words) (Turkish and English)

• Keywords (minimum 3; maximum 6)

• Article divided into appropriate sections

• Complete and accurate references and citations

• List of references styled according to "journal requirements"

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RESEARCH ARTICLES

Research Article

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Viral Hepatitis Journal 2020;26(2):43-48

Evaluation of Treatment Results with Direct Acting Antiviral Drugs of Cirrhotic/Non-cirrhotic Chronic Liver Disease Caused by Hepatitis C Virus Genotype 1b Infection

Hepatit C Virüs Genotip 1b Kaynaklı Sirotik/Sirotik Olmayan Kronik Karaciğer Hastalığı Olgularında Doğrudan Etkili Antiviral İlaç Tedavisi Sonuçlarının Değerlendirilmesi

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ABSTRACT

Objectives: This study aimed to investigate the effect of treatment with direct-acting antivirals (DAAs) on the virological response and on the some parameters used to evaluate liver function in cases with chronic liver disease due to hepatitis C virus (HCV) genotype 1b.

Materials and Methods: This study included cases who were treated with DAAs after HCV genotype 1b infection. HCV-RNA levels and biochemical and hematological parameters measured at the beginning of treatment, 12th week and 52th week after the treatment were transferred to the SPSS statistics software. model for end-stage liver disease (MELD) and Child-Pugh scores were also calculated and added to these data.

Results: The study group consisted of a total of 102 patients, including 33 (32%) males and 69 (68%) females. Compensated cirrhosis was detected in 26.5% of the patients (n=27). There was a significant change in serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alpha-fetoprotein (AFP) parameters in patients with compensated cirrhosis after treatment, and total bilirubin, hemoglobin, ALT, AST, GGT, ALP and AFP parameters in the group without cirrhosis (p<0.05). Only a significant decrease was observed in the MELD score of the patients with compensated cirrhosis (p=0.007).

Conclusion: The ombitasvir/paritaprevir/ritonavir+dasabuvir and ledipasvir/sofosbuvir regimens are very effective and safe in the treatment of patients who develop chronic liver disease and compensated liver cirrhosis after HCV genotype 1b infection.

Keywords: HCV, PrOD, MELD, Child-Pugh, compansated cirrhosis

ÖZ

Amaç: Bu çalışmanın amacı, hepatit C virüs (HCV) genotip 1b'ye bağlı kronik karaciğer hastalığı gelişen olgularda, doğrudan etkili antiviral (DEA) ilaçlar ile yapılan tedavinin virolojik yanıt ve karaciğer fonksiyonlarını değerlendirmek için kullanılan bazı parametreler üzerine etkisinin irdelenmesidir.

Gereç ve Yöntemler: Retrospektif bir çalışmadır. Bu çalışmaya HCV genotip 1b enfeksiyonu sonrası DEA ilaçlar ile tedavi edilen 18 yaşından büyük olgular dahil edildi. Tedavi başlangıcı, 12. ve 52. haftalara ait HCV-RNA düzeyi, biyokimyasal ve hematolojik parametreler SPSS istatistik programına aktarıldı. Bu verilere, son dönem karaciğer hastalığı için model (MELD) ve Child-Pugh skorları da hesaplanarak eklendi.

Bulgular: Çalışma grubu 33'ü (%32) erkek, 69'u (%68) kadın 102 hastadan oluşmaktadır. Hastaların %19'unda (n=20) kompanse siroz saptandı. Tedavi sonrası kompanse sirozu olan hastalarda serum albümin, alanin aminotransferaz (ALT), aspartate aminotransferas (AST), gama glutamil transferaz (GGT) ve alfa Fetoprotein (AFP) parametrelerinde, sirotik olmayan grupta ise total biluribin, hemoglobin, ALT, AST, GGT, ALP ve AFP parametrelerinde anlamlı değişiklik saptandı (p<0,05). Siroz olmayan hastaların MELD ve Child skorlarının puan değeri tedavi sonrası azalmakla birlikte anlamlı değişiklik olmadı. Kompanse sirozu olan hastaların ise yalnız MELD skorunda anlamlı bir azalma (p=0.007) saptandı.

Sonuç: Ombitasvir/paritaprevir/ritonavir+dasabuvir ve sofosbuvir/ledipaspir rejimleri, HCV genotip 1b enfeksiyonundan sonra kronik karaciğer hastalığı ve kompanse karaciğer sirozu gelişen hastaların tedavisinde çok etkili ve güvenlidir.

Anahtar Kelimeler: HCV, PrOD, MELD, Child-Pugh, kompanse siroz

Doğan M, Topçu B, Karaali R, Erdem İ. Evaluation of Treatment Results with Direct Acting Antiviral Drugs of Cirrhotic/Non-cirrhotic Chronic Liver Disease Caused by Hepatitis C Virus Genotype 1b Infection. Viral Hepat J. 2020;26:43-48.

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Introduction

Hepatitis C virus (HCV) is known as the single-stranded, enveloped, smallest RNA virus of the Flaviviridae family. It is divided into at least six groups and many subtypes according to its genotype. Although different rates are reported regionally in different studies reported from Turkey, the most frequently seen genotype is 1b (1). Chronic HCV infection is one of the most important causes of chronic liver disease, hepatocellular carcinoma, and cirrhosis. More than 185 million people worldwide are thought to be infected with HCV and more than 85.000 people in Turkey (1). About 60-85% of these cases become chronic (2). An effective treatment is of great importance in terms of breaking the infection chain, preventing the spread and reducing the morbidity and mortality caused by the virus. With the introduction of direct-acting antivirals (DAAs), a sustained virologic response (SVR) of up to 99% has been achieved in the treatment of these patients, leading to the beginning of a new period (3). A limited number of cases have been reported to discontinue treatment due to side effects and unforeseen causes during the treatment. However, these drugs provided satisfactory results in the follow-up of the disease with their ease of use, easy tolerance and low side effect profiles (4). This study aimed to investigate the treatment results obtained with DAAs in patients with HCV genotype 1b and the effect of this treatment on some laboratory parameters evaluating liver damage and on the score values obtained from the scoring methods.

Materials and Methods

This is an observational study aimed at collecting retrospective data. This study was carried out with the approval of Ethical Committee of Namık Kemal University Faculty of Medicine (approval number 2020.86.04.10). The procedures were performed in accordance with the Declaration of Helsinki. Since our study was retrospective, informed consent was not used. This study covered the date range of 01.01.2016-31.12.2019. Patients older than 18 years of age, who were evaluated in our outpatient clinic within the specified date range, had anti-HCV positivity and received DAA medication, were included. The study group consisted of 102 cases. Demographic data, serological data, sustainable virological response, treatment regimen and side effects were transferred to the study form. The HCV-RNA values measured before the treatment, end of the treatment and 52th week, as well as biochemical and hematological analysis results, were also recorded in the study form. The model for end-stage liver disease (MELD) and Child-pugh scores of these patients were also calculated and transferred to the form.

Statistical Analysis

Data were transferred to the study form and analyzed using SPSS statistical software. Variables were expressed in frequency, percentage, mean, standard deviation, table, and graph. The normality test was performed and all variables were seen to follow a normal distribution. Paired Samples test was used to compare the pre- and post-treatment values of the continuous variables. A p-value of <0.05 was considered statistically significant.

Results

Of the 153 patients, 15% (n=23) were seen not to come to regular polyclinic controls and 18% (n=28) were other genotypes. The study group consisted of 102 patients with genotype 1b. The mean age of these patients was 59.43±14 years (minimum: 21, maximum: 83). Of the cases, 32% (n=33) were male and 68% (n=69) were female. Of the patients, 82% (n=84) were naive and 17% (n=17) previously received pegylated interferon plus ribavirin treatment and one case received boceprevir with peginterferon alfa-2a-ribavirin treatment. Compensated cirrhosis diagnosis was observed in 26.5% of the patients (n=27). In the treatment of 82% of patients, the ombitasvir/paritaprevir/ritonavir tablet 12.5/75/50 mg once a day two tablets at the same time and dasabuvir (PrOD) tablet 250 mg twice daily regimen was seen to be used whereas Sofosbuvir/ledipasvir 400/90 mg once a day regimen was used in the treatment of 18%. Demographic characteristics and clinic parameters of patients before treatment are shown in Table 1.

Table 1. Demographic characteristics and clinic parameters of patients

Characteristic	n (%)
Age	59.43±14
Sex	
Female	69 (68)
Male	33 (32)
Genotype	
Genotype 1b	102 (100)
Treatment history	
Naiv	84 (82.4)
Pegylated interferon/ ribavirin	17 (16.7)
Bocepravir + pegylated interferon alfa 2A/ribavirin	1 (0.09)
Liver disease	
No cirrhosis	75 (73.5)
Compensated cirrhosis	27 (26.5)
Antiviral treatment in patients without cirrhosis	
Ombitasvir/paritaprevir/ritonavir + dasabuvir	57 (76)
Sofosbuvir/ledipasvir	18 (24)
Antiviral therapy in patients with compensated cirrhosis	
Ombitasvir/paritaprevir/ritonavir+ dasabuvir	26 (96)
Sofosbuvir/ledipasvir	1 (4)
HAI	
No cirrhosis	7.2±2.1
Compensated cirrhosis	10.1±1.8
HCV-RNA level	
No cirrhosis	2235654,094±4707658,56 IU/mL (minimum: 675, maximum: 31437735)
Compensated cirrhosis	737205,53± 388069,64 IU/mL (minimum: 79127, maximum: 5177768)

HAI: Hepatitis activity index, HCV: Hepatitis C virus

There was no statistically significant difference in terms of treatment success in both groups (p>0.05). In 99% of the patients (n=101), HCV-RNA levels were found below the determinable level in the fourth week of treatment. The SVR was found to be 99% at 12 weeks after treatment. In the evaluation of a case where no virological response was seen, it was learned that the patient was anti-human immunodeficiency virus (HIV) positive, received antiretroviral therapy (elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil), and was using an intravenous agent. In the evaluation made at the 52th week after treatment, recurrence (HCV-RNA: 4277 IU/mL) was detected in one case, while SVR maintained in other cases. No detectable risk factor was found in the recurrent case. No patient discontinued the treatment due to adverse effects. There was no relationship between advanced age [≥65 years (n=47), <65 years (n=55)] and treatment success among the cases.

Considering the pre- and post-treatment laboratory parameters, there was a significant change in serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alpha-fetoprotein (AFP) parameters in patients with compensated cirrhosis after treatment, and total bilirubin, hemoglobin, ALT, AST, GGT, ALP and AFP parameters in the group without cirrhosis (p<0.05). The MELD and CHILD scores of patients without cirrhosis were seen to decrease after treatment, but this decrease was not significant. However, there was a significant decrease in the MELD score of patients with compensated liver cirrhosis (p=0.007). Descriptive statistics of surveyed variables among patient are shown in Table 2.

Discussion

This retrospective single-center study consists of real-life data obtained between 2016-2019. Chronic HCV infection is one of the most important causes of cirrhosis and related liver diseases. The SVR achieved following an effective treatment significantly reduces morbidity and mortality even in advanced fibrosis (5). High levels of success have been achieved via both PrOD-based and sofosbuvir-based treatment regimens in the treatment of cases with HCV genotype 1b (6). The SVR rate has been reported to vary between 84% and 100% depending on patient groups and risk factors (7,8). The presence of liver cirrhosis stands out as an independent risk factor affecting SVR-12 (6). However, high SVR-12 can be achieved regardless of the liver cirrhosis stage (9). Treatment success (SVR-12) has been found to be higher in patients with albumin >3.5 g/dL, bilirubin <2 mg/dL and Child-pugh scores score 5-6 in the presence of liver cirrhosis (10). Furthermore, in a different evaluation, 100% SVR-12 was obtained with the PrOD regimen in the group, where 98.4% of patients had a Child-pugh score of 5 points, and no side effects that could lead to the discontinuation of treatment were observed (11).

Progression to decompensation can be seen in patients with compensated cirrhosis during the PrOD-based treatment regimen (12). There are some risk factors that facilitate decompensation. The main two predictive factors in progression to decompensation have been reported to be advanced age (>65 years) and albumin level of <3.6 g/dL (13). The development of hyperbilirubinemia during the treatment has been reported to be another facilitating factor (13). Furthermore, the rate of progression to decompensation in patients with compensated cirrhosis varies widely among patient groups. Progression to decompensation was observed in 18.52% of the patients with compensated cirrhosis, who developed hyperbilirubinemia during the PrOD regimen (13). In contrast, there are studies indicating that decompensation may develop at the rate of 2% in patients with compensated cirrhosis treated with a PrOD-based regimen, however, this treatment cannot be associated with mortality (12). The possibility of hepatocellular carcinoma development has been reported to be 1.4% in the follow-up period of these cases (10,12).

Table 2. Descriptive statistics of surveyed variables among patient

Variables	Compensated cirrhosis		p	Non cirrhosis		p
	Median ± SD			Median ± SD		
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
Serum albumin (gm/dL)	3.87±0.49	4.22±0.66	0.001	4.32±0.47	4.27±0.51	0.358
Total bilirubin (mg/dL)	0.70±0.39	0.66±0.37	0.623	0.64±0.45	0.49±0.35	0.000
ALT (IU/L)	40.59±24.55	17.77±7.22	0.000	49.07±32.88	13.94±7.27	0.000
AST (IU/L)	43.62±25.94	19.59±8.86	0.000	43.91±34.95	16.91±6.20	0.000
γGT (IU/L)	47.04±36.82	28.00±22.26	0.004	55.01±52.28	22.29±21.17	0.000
ALP (IU/L)	95.59±31.36	92.09±34.06	0.409	112.18±55.56	98.89±39.96	0.015
WBCs (x10^9/L)	6.73±2.51	6.78±2.62	0.815	7.03±4.16	7.20±4.47	0.301
Platelets (x10^9/L)	187.80±94.59	186.38±89.55	0.884	220.95±78.83	228.41±87.78	0.133
Hemoglobin (gm/dL)	13.21±2.23	13.10±2.01	0.568	13.29±1.93	12.35±2.21	0.000
PT (Second)	15.16±2.51	15.01±1.90	0.687	14.49±2.85	14.08±2.50	0.057
INR	1.12±0.16	1.08±0.12	0.123	1.06±0.18	1.06±0.15	0.976
AFP (IU/mL)	9.34±14.70	3.52±2.14	0.045	5.71±4.97	3.01±2.55	0.000
MELD	11.44±6.97	11.04±6.85	0.007	8.00±3.27	7.53±2.91	0.069
Child-Pugh	5.46±0.85	5.15±0.36	0.043	5.10±0.52	5.07±0.31	0.484

SD: Standard deviation, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, γGT: Gamma-glutamyl-transferase, ALP: Alkaline phosphatase, WBC: White blood cells, PT: Prothrombin time, INR: International normalization ratio, AFP: Alpha feto protein, MELD: Model for end-stage liver disease

It has been demonstrated that there is no difference between the sofosbuvir-based regimen and the PrOD-based regimen in terms of treatment success in patients with genotype 1 in the presence of compensated liver cirrhosis (8). In the present study, no difference has been observed between the sofosbuvir-based regimen and the PrOD-based regimen in terms of SVR-12.

Advanced age is considered as a condition that may affect SVR success. In the HCV genotype 1 cases, the post-treatment SVR-12 rates have been reported to be 94% and 100% in patients aged ≥65 years and <65 years, respectively (14). However, PrOD regimen has been reported to be effective and reliable in patients aged ≥65 years. In the present study, no statistically significant difference has been observed between the patients aged >65 years and <65 years in terms of SVR-12.

In a study comparing PrOD ± RBV treatment results of cases with HIV/HCV coinfection and cases with HCV infection alone, a 2.2% difference was observed in terms of SVR-12, but no statistically lower difference was found. In cases with coinfection alone, HCV genotype 4 has been found to be associated with non-response to treatment (15). In another study, the results of PrOD ± RBV treatment in genotype 1 and 4 patients with coinfection were compared and the results were seen to be similar; SVR-12 was achieved at a rate of 97.8% and 97.6%, respectively (9). When the results of two patients with HIV/HCV coinfection included in the present study were evaluated, no virological response to DAA treatment was observed in a patient with simultaneous IV drug use.

In a study comparing eight-week and 12-week treatment periods in patients with genotype 1b, who were treated with the PrOD regimen, SVR was achieved at a rate of 95% and 99% after eight-week and 12-week treatments, respectively and no factor related to treatment non-response was found (16). The virus may be re-detected in some cases during the HCV-RNA follow-ups after SVR. The recurrence rate is reported to be about 1% (7,17). Recurrence was observed in one of our patients at the 52th week follow-up following the achievement of SVR. No etiological reason associated with recurrence was found.

High virological response success can also be achieved in patients who have had unsuccessful treatment experience with DAA treatment (18). A 100% SVR-12 has been achieved with the PrOD ± RBV regimen in compensated cirrhotic cases, about 70% of whom have treatment experience.

Mild and moderate adverse effects may occur in patients receiving PrOD ± RBV therapy. In particular, fatigue, headache, sleeplessness, itching, diarrhea and anemia have been reported more frequently (19). No toxic changes related to DAA treatment have been observed in laboratory parameters. Furthermore, no cases where the treatment was discontinued due to adverse effects were reported (11). However, severe adverse effects that may cause discontinuation of treatment may develop (6). In the present study, the most common adverse effect was itching and there were no adverse effects causing discontinuation of the treatment or requiring additional treatment.

Treatment of chronic liver disease with DAA can affect the physical and mental scores of the patients. Positive changes can occur in social lives in particular. More cost-effective changes can be seen in the quality of life and conditions of patients after treatment (20).

Treatment success has been found to have no significant relationship with the age, gender, previous treatment, body mass index, platelet count, international normalized ratio, and MELD score (13). However, the MELD score of <10 and the ALT value of 20 U/L in the 8th week of the treatment have been demonstrated to be positive markers for the virological response (21).

Some biochemical parameters that are above the reference range before treatment may return to normal limits after DAA treatment. Moreover, white blood cell count, platelet count, and hemoglobin values may change during and after treatment. These changes have been reported in both cirrhotic and non-cirrhotic cases. Significant changes can be seen in ALT, AST, GGT, ALP, platelet count, serum albumin and total bilirubin values following the DAA treatment (8,10,21,22). In the literature, there are also studies reporting that there is no significant difference in the white blood cell, hemoglobin, and platelet count (22). In the present study, a significant change has been observed in albumin, ALT, AST, GGT and AFP parameters in cirrhotic patients and total bilirubin, ALT, AST, GGT, ALP, AFP and hemoglobin parameters in non-cirrhotic patients after the treatment ($p<0.05$).

There are scoring criteria used to assess the level of liver damage and the well-being of the patient. It is thought that DAA treatment may lead to positive changes in these criteria and decrease the fibrosis score, resulting in a reduction in the burden of disease. When the initial and post-treatment first-year MELD scores and degree of fibrosis measured using FibroScan were evaluated, a significant change has been observed in both parameters ($p<0.05$) (10). Furthermore, it has been seen that significant changes may occur in the CHILD score and physical life score of patients following the DAA treatment (10). Contrary to this, progression to decompensation is seen in very few patients with compensated cirrhosis (23). In the present study, a decrease has been observed in the post-treatment MELD and Child-pugh scores of patients with compensated cirrhosis compared to the pre-treatment scores, but there were no significant changes. However, a significant decrease has been seen in the MELD scored of patients without cirrhosis ($p=0.007$).

Study Limitations

The main limitation of our study is that it has a retrospective design. In addition, subgroup analysis was not performed based on the accompanying risk factors, HCV-RNA levels, and histological activity indices.

Conclusion

This study consists of cases with HCV genotype 1b and chronic liver disease. Regardless of age, gender, viral load, and underlying diseases, a high level of SVR has been achieved in all cases included in the study. Furthermore, returning to normal limits has been observed in indirect markers used to determine the level of liver damage. The retrospective design of this study is its weakness. However, we believe that very valuable data have been presented thanks to the results it has revealed.

Ethics

Ethics Committee Approval: This study was carried out with the approval of Ethical Committee of Namık Kemal University Faculty of Medicine (approval number: 2020.86.04.10).

Informed Consent: Since our study was retrospective, informed consent was not used.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.D., B.T., R.K., İ.E., Concept: M.D., B.T., R.K., İ.E., Design: M.D., B.T., R.K., İ.E., Data Collection or Processing: M.D., B.T., R.K., İ.E., Analysis or Interpretation: M.D., B.T., R.K., İ.E., Literature Search: M.D., B.T., R.K., İ.E., Writing: M.D., B.T., R.K., İ.E.

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Research Article

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Hepatitis C Virus Genotype Distribution in Forensic Cases

Adli Olgularda Hepatit C Virüs Genotip Dağılımı

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ABSTRACT

Objectives: In this study, we aimed to determine the hepatitis C virus (HCV) genotype and subtypes in blood samples that were determined by polymerase chain reaction (PCR) in autopsy cases.
Materials and Methods: The blood samples of autopsy cases that was sent to serological screening to post-mortem microbiology laboratory between years 2014-2018 were recruited. Forty blood HCV-PCR positive autopsy cases were further evaluated including demographic, clinic, laboratory and autopsy features.
Results: Thirty-five 35 (87.5%) of the patients were male and 5 (12.5%) were female. The mean age of the patients was 43.1±11.8 years. Of the 40 cases, 18 (45%) were Turkish citizens and 16 (40%) were other nationals. The identity information of 6 cases (15%) could not be determined. Among 40 HCV-positive cases by PCR, the genotype 3 was determined in 11 (27.5%) of the cases, genotype1a in 9 (22.5%) cases, genotype-1b in 7 (17.5%) cases, genotype-2 in 2 (5%) cases and genotype-4 in 2 (5%) cases. In 9 (22.5%) cases, the genotype could not be determined.
Conclusion: The most common HCV genotype in our study population was determined to be genotype-3 and the most common genotype in Turkish origin cases was found to be genotype-1a. Post-mortem PCR analysis for HCV infection is feasible and relevant for demonstrating the ongoing infections at death. Monitoring the change in HCV genotype distribution is critical for the development of effective strategies for HCV elimination.
Keywords: Hepatitis C Virus, genotype, molecular epidemiology, post-mortem microbiology

ÖZ

Amaç: Bu çalışmada, otopsi olgularından polimeraz zincir reaksiyonu (PCR) ile hepatit C virüs (HCV) pozitif saptanan kan örneklerinde HCV genotip ve alttiplerinin belirlenmesi amaçlanmıştır.
Gereç ve Yöntemler: 2014-2018 yılları arasında post-mortem mikrobiyoloji laboratuvarına serolojik tarama için gönderilen otopsi olgularının kan örnekleri alındı. Demografik, klinik, laboratuvar ve otopsi özellikleri de dahil olmak üzere 40 HCV-PCR pozitif otopsi olgusunun kan örnekleri değerlendirildi.
Bulgular: Çalışmaya alınan olguların 35'i (%87,5) erkek, 5'i (%12.5) kadın olup, olguların yaş ortalaması 43,1±11,8 yıl olarak belirlenmiştir. 40 olgunun 18'i (%45) Türk vatandaşı olup, 16'sı (%40) yabancı uyrukludur. Altı (%15) olgunun da kimlik bilgilerine ulaşılamamıştır. Real time PCR analiz sonuçlarına göre örneklerin 11'inde (%27,5) genotip 3, 9'unda (%22,5) genotip 1a, 7'sinde (%17,5) genotip 1b, 2'sinde (%5) genotip 2 ve 2'sinde (%5) genotip 4 tespit edilmiştir. Örneklerin 9'unda (%22,5) ise genotip tayini yapılamamıştır.
Sonuç: Çalışmamızda, genel popülasyonda en sık HCV genotip 3 saptanırken, Türk vatandaşlarında ise en sık saptanan genotip 1a olmuştur. HCV enfeksiyonu için post-mortem PCR analizi uygulanabilir ve ölümde devam eden enfeksiyonları göstermek için önemlidir. HCV genotip dağılımındaki değişikliğin izlenmesi, HCV eliminasyonu için etkili stratejilerin geliştirilmesi için kritik öneme sahiptir.
Anahtar Kelimeler: Hepatit C Virüs, genotip, moleküler epidemiyoloji, post-mortem mikrobiyoloji

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Introduction

Hepatitis C has been a global health problem with a well-known importance since the identification of the Hepatitis C virus (HCV) in 1989. During the last 15 years seroprevalance of HCV has been increasing and more than 185 million people are tought to be infected with this virus all around the world (1,2,3). Initially, HCV was thought to be the most common cause of post-transfusion

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related hepatitis and almost not changing the life expectancy in infected individuals but later on studies shown that 85% of the HCV cases become chronic and HCV is one of the most common cause of deaths due to liver cirrhosis and the reason for liver transplantation. In our country, HCV is the second most common cause of chronic viral hepatitis after Hepatitis B virus (4).

After the full-length genome sequences of HCV strains up to date 7 genotype and more than 100 subtypes have been defined based on on phylogenetic and sequence analyses (5,6). HCV genotypes are expressed in numbers from 1 to 7 and subtypes are expressed in lowercase letters such as a, b, c, etc. Each HCV genotype differs from each other by at least 20% at the nucleotide level and by more than 15% at the amino acid level (6). Moreover, base sequence changes within the same genotype can be observed at a rate of 5-8% in the nucleotide sequence and at a rate of 4-5% in the amino acid bases. The global geographical distribution of HCV genotypes is various. Some genotypes appear to be seen in a particular region of the world. In North America, genotype 1a predominates, whereas genotype 1b, which is more commonly associated with aggressive liver disease, is more common in Western Europe and Japan. Genotype 2 is less common in Europe than Asian countries such as China, Japan and Taiwan. Genotype 3 is usually found in the UK and Thailand, while genotype 4 is seen in Middle East region and Central Africa. Genotype 5 is observed in South Africa and genotype 6 in Hong Kong (7). Geneotype 7 has been reported to from Democratic Republic of Congo in Central Africa (6,8). The most common observed genotype in Turkey is the genotype 1b, followed by genotype 1a (9,10). Besides 7 genotypes, there are 67 confirmed and 20 possible subtypes of HCV identified with at least 15% genomic variation; genotypes 5 and 7 have a single subtype, while at least seven subtypes are observed in other genotypes (11).

The most important problem of post-mortem serological analysis is that its validity. Higher positive results was observed in some studies more than expected (12,13,14). For this reason, it is recommended to use sensitive kits for post-mortem serological examinations (15). Unfortunately, most of the kits used in routine microbiology laboratory for post-mortem serological examinations were not validated for post-mortem samples. Due to loss of specific reactions, the post-mortem samples show decreased sensitivity and increased false-negative results (15). For these reasons, anti-HCV antibody tests was not used in the blood samples of autopsy cases. The success of post-mortem microbiological investigations depends on the adequacy of the post-mortem sampling protocol and strategy (16). Firstly, it is recommended to perform the autopsy preferably within the first 24 hours after death. Microbiological samples should be obtained as soon as possible. In particular, it is emphasized that blood samples should be drawn at the beginning of autopsy (17). As a general principle, the samples should be obtained at room temperature as soon as within 2 hours, stored in suitable transport and storage environments, and if samples was needed to transferred to the study laboratory should be done within 48 hours at 2 to 8 °C (18). The most common drawbacks of post-mortem serological and molecular examinations occurs because of insufficient quantity or bad quality of blood samples, or samples obtained at inappropriate time intervals (19,20).

The aim of this study was to determine the distribution of HCV genotypes and subtypes in blood samples that were determined by polymerase chain reaction (PCR) in autopsy cases in Turkey.

Materials and Methods

This study was done in Turkish Ministry of Justice Council of Forensic Medicine Post-mortem Microbiology Laboratory, in İstanbul. Forty HCV positive autopsy cases were included in this study between years 2014 to 2018. This study was approved by the Ethics Commitee and Research and Scientific Research Commission of the Ministry of Justice Council of Forensic Medicine (approval number: 21589509/2019/125).

Blood samples were drawn from large vessels (femoral artery, femoral vein, jugular vein) for serological and PCR evaluations of autopsy cases. The blood samples were transferred to EDTA tubes and sent to the laboratory as fast as possible. After centrifuging the post-mortem blood samples at 10.000 rpm for 10 minimum, the resulting plasma samples were aliquoted and stored at -80 °C until the assay. 400 µl of the plasma samples were removed and RNA isolation was performed on the QIA symphony device with the QIA symphony DSP Virus/Pathogen Midi kit, and the amplification of the RNA was performed on the Rotor-Gene® Q device (Qiagen, Germany) by the RT PCR method using the Artus® HCV-PCR kit. For determination of the HCV genotypes (RTA) HCV Genotyping qPCR Kit [targeting NS5b and NS3 of viral 5'-UTR region obtained by real time (RT)-PCR] (RTA, Kocaeli, Turkey) was used. The Kit identifies the six major and most common HCV genotypes (1, 1a, 1b, 2, 3, 4, 5, 6). Analysis was carried out on the CFX C1000 Touch instrument (Bio-Rad, Hercules, USA) and genotypes were determined.

Statistical Analysis

The statistical software program of SPSS (Version 16) was used in the data analysis of the study. The descriptive statistics were expressed as numbers and percentages.

Results

Of the 40 cases included in the study, 35 (87.5%) were male and 5 (12.5%) were female, and the mean age was 43.1±11.8 years (range: 23-69 years). Among the 40 cases, 18 (45%) were Turkish citizens and 16 (40%) were foreign nationals. We could not reach nationality of 6 (15%) cases (Table 1). Demographic features, HCV genotype distribution, clinical history, laboratory results and the autopsy results of the study population were shown in Table 2.

Table 1. The distribution of the autopsy cases according to nationality

	Number of cases	%
Turkish	18	45
Turkmenistan	4	10
Georgia	3	7.5
Pakistan	3	7.5
Syria	2	5
Uzbekistan	2	5
South Africa	1	2.5
Tanzania	1	25
Unknown	6	45
Total	40	100

Table 2. Demographic, clinical, laboratory and autopsy results of the study cases

	Gender	Age	HCV genotype	Clinical history	Co-morbidity status	Autopsy results
1	Male	35	1a	HCV positive, cocaine intoxication, intensive care unit admission, intubation due to low glascow coma score and respiratory distress,	-	Death due to drug intoxication.
2	Male	38	2/1a	Hospitalized with unconsciousness, death after hospitalization.	Pulmonary tuberculosis	Death due to multiple drug intoxication and complications.
3	Female	47	1b	Hospitalized with the complaint of abdominal pain a day before, sent after the intervention and died in next day.	Cirrhotic liver, hepatic encephalopathy	Death due to peritonitis and complications.
4	Male	43	3	Death at home	-	Death due to drug intoxication.
5	Male	38	3	History of drug intake, taken to emergency room by the foreigners' office, death after hospitalization	-	Sent to 1th forensic expertise board of the council of forensic medicine
6	Male	29	1a	Death after hospitalization	-	Death due to drug intoxication.
7	Male	38	1a	Found dead on the street	-	Death due to drug intoxication.
8	Male	25	1b/3	Admission to the emergency room after fainting on the street, respiratory distress, intensive care unit admission	-	Death due to Lung Infection and related complications and due to drug intoxication.
9	Male	64	1a	Chronic schizophrenia, 34-40% body burn due to fire in hospital ward	-	Death due to body burn and related complications.
10	Male	36	1b	Hospitalized on the deterioration of the general condition, death after hospitalization.	-	Death due to drug intoxication.
11	Male	56	3	Operated for cerebral hemorrhage due to hypertension, 15 days intensive care unit stay	-	Death due to Non-traumatic cerebral hemorrhage and developing complications
12	Male	52	1a	Tuberculosis, 2 months anti-TBC treatment, complained of chills, chest pain, hospitalization due to deterioration of the general condition	Tuberculosis	Lung infection and died due to drug intoxication
13	Male	37	2	Drug intake? Hospitalization due to unconsciousness, exitus	-	Death due to drug intoxication and complications
14	Male	25	N/A	Hospitalized on the deterioration of the general situation at home, drug intake?	-	Death due to drug intoxication and complications
15	Male	37	3	Treatment for substance abuse, hospitalization due to deterioration of the general condition at home	-	Death due to drug intoxication.
16	Male	28	2/1b	Found dead on the street	Growth of *Streptococcus pyogenes* in blood, lung, spleen, and pleural fluid	Died due to systemic infections and drug intoxications
17	Female	58	3	Death at the care center	DM, cirrhosis, COPD	Death due to Self-existing disease (DM, cirrhosis, COPD) and complications
18	Female	31	1b	One month ago intensive care unit admission due to traffic accident, ARDS, exitus	-	Sent to 1st forensic expertise board of the council of forensic medicine
19	Male	45	3	No history of disease before, admitted to the emergency department after the use of bonzai, unconscious, intubated, 1 week ICU stay.	-	Death due to drug intoxication.
20	Male	35	4	Found dead on the street	HIV positivity	Death due to drug intoxication and complications

Table 2. contiuned

21	Male	63	1a	Hospitalization due to non-vehicle traffic accident, operated due to hypertension, ICU admission	-	Died due to trauma to the general body, brain hemorrhage and complications.
22	Female	65	N/A	Body burn due to fire at home	-	death due to body burn and related complications (lung infection, sepsis).
23	Male	55	N/A	Death at home	-	Death due to blunt head trauma and brain hemorrhage
24	Male	33	N/A	Three weeks hospitalization after gunshot injury	-	Death due to Spinal cord injury, gunshot injury and spinal cord injury related complication
25	Female	44	1b	Hospitalized on the deterioration of the general condition, death after 3 day hospitalization.	-	Methyl alcohol intoxication and related complications
26	Male	45	3	Found dead on the street	-	Death due to drugs and inhalant intoxication.
27	Male	52	3	Hospitalized on the deterioration of the general condition, death after hospitalization, drug intake?	-	Death due to gastrointestinal bleeding and gastric ulcer complications and drug intoxication,
28	Male	52	3	Death at home, liver cirrhosis and cancer.	Cancer, liver cirrhosis	Death due to cirrhosis and related complications.
29	Male	69	1a	A history of subarachnoid hemorrhage after a fall from stairs, post op exitus.	-	Sent to 1th forensic expertise board of the council of forensic medicine
30	Male	42	N/A	A history of AIDS and drug abuse, death in police custody.	HIV positivity	Death due to AIDS disease and related complications.
31	Male	50	2/1b	Death at home.	-	Sent to 1st forensic expertise board of the council of forensic medicine.
32	Male	45	1b	Drug intake. Found wounded roadside with unconscious, death after hospitalization,	Cardiovascular disease	Death due to methyl alcohol intoxication.
33	Male	34	3	Hospitalized after falling, 1 week ICU stay.	-	Died of skull damage, facial bone fractures, brain hemorrhage, and brain tissue damage due to traumatic body trauma.
34	Male	23	4	Bronchitis history, hospitalized on the deterioration of the general condition at home	Bronchitis	Sent to 8th forensic expertise board of the council of forensic medicine
35	Male	28	1a	Prisoner, death outside the prison when he was on leave.	-	Death due to drug intoxication.
36	Male	58	1b	Heart attack at home, death after hospitalization	-	Death due to cardiovascular disease.
37	Male	44	3	Found dead on the street, had drugs in pocket	-	Death due to lung infection and cardiovascular disease.
38	Male	37	1a	Found dead on the street, history of COPD, alcohol and drug usage,	HBV positivity	Death due to pulmonary tuberculosis and its complications
39	Male	41	1b	Death after 15 day hospitalization.	Tuberculosis culture and Tbc-PCR positivity in lung, spleen, and HBV-HIV positivity	Death due to systemic tuberculosis and related complications
40	Male	50	2	Found dead on the street	-	Death due to drug intoxication.

*N/A could not be genotyped.
HCV: Hepatitis C virus, DM: Diabetes mellitus, COPD: Chronic obstructive pulmonary disease, ICU: Intensive care unit, ARDS: Acute respiratory distress syndrome, PCR: Polymerase chain reaction, HIV: Human immunodeficiency virus

By using the RT-PCR, HCV genotype 3 was determined in 11 (27.5%) of the samples, HCV genotype 1a in 9 (22.5%) samples, HCV genotype 1b in 7 (17.5%) samples, HCV genotype 2 in 2 (5%) samples, and HCV genotype 4 in 2 (5%) samples. HCV genotype could not be determined in 9 (22.5%) samples. The most common HCV genotype in the study cases was genotype 3, while the most common HCV genotype in Turkish citizens was genotype 1a. The genotype distribution of Turkish citizens and foreign nationals was shown in Table 3.

We found that 22 (55%) autopsy cases had a history of intravenous drug usage. All of the 22 cases were male, 10 of them were Turkish citizens. The HCV genotype distribution of 22 samples was as follows: genotype 3 in 7 (31.8%), genotype 1a in 6 (27.3%), genotype 2 in 2 (9.1%), genotype 1b in 1 (4.5%) and genotype 4 in 1 (4.5%) sample. The genotype could not be determined in 5 samples (22.7%). Among the 10 Turkish citizens with history of intravenous drug usage; genotype 1a was found in 5 (50%) of them, genotype 3 in 2 (20%) of them, and genotype 1b in 1 (10%) of them, and genotype could not be detected in 2 (20%) of them.

Study Limitations

There are also several limitations to our study, the rate of genotype 3 was found to be which is higher than the rates reported previously from our country. So, it is not possible to reach a definitive conclusion due to the low number of cases in our study. The second limitation, in our study, we used an automated PCR based method. Although the RT-PCR method has advantages such as being user independent, standard, automatic and yielding fast results, but in our study, the genotype of HCV subtypes could not be determined in 22.5% of cases.

Conclusion

The most common detected HCV genotype has been reported to be genotype 1, at a rate of 46% all around the world (21). In our country, while the most common HCV genotype in hepatitis C patients between years 1995 and 2014 was found to be genotype 1b (22,23), but most recently, genotype 3 was found to be the most common genotype in intravenous substance addicts, and in prisoners in different two studies (24,25). Although genotype 3 has been reported at low rates (0-4.5%) in studies conducted in different centers in Turkey, after year 2010, genotype 3 detection rates has been increasing significantly (26). In our study, the most common genotype detected in study samples was genotype 3 (27.5%), and the genotype 1a (44%) took the first place in samples from Turkish citizens. Our findings show that the presence of foreign nationals in the HCV genotype distribution conflicts with our country data. Events that cause social changes such as war, migration and tourism affect the epidemiology of infections (23). In our study, the heterogeneity among genotypes may be related to demographic changes in our country due to its geographical location. We thought that the presence of high number of foreign nationality in our cases may be responsible for excessive detection of genotype 3 (27.5%). On the other hand, the rate of genotype 3 was found to be 22.2% in Turkish nationality cases which is higher than the rates reported previously from our country (27,28). Although it is not possible to reach a definitive conclusion due to the low number of cases in our study, it may be concluded that genotype 3 is becoming more common in our country. The main route of transmission of HCV is parenteral. After initiation of screening programs in blood and blood products, intravenous drug usage has become the main parenteral route of transmission. In European countries such as France, Germany, Italy and Sweden 30-59% of all HCV infections are associated with intravenous drug usage. In America, this rate reaches 68% (29). The anti-HCV antibody positivity prevalence among intravenous drug users in Turkey has been reported to be 28.9% (30). In another study, HCV infection was determined in 47% of intravenous drug users that were mostly adolescents and young adults (31). In addition, the lack of attention to sterilization and disinfection during the medical procedures is also one of the most common causes of HCV transmission in our country (32). In a study conducted in our country in young people with intravenous drug addiction, the most common genotype was determined to be genotype 1a (26). In our study, 22 (55%) patients had a history of intravenous drug use and all were male. Ten (45%) of these drug users were Turkish citizens. In our cases among the IV drug users genotype 3 (7/22; 31.8%) was found to be the most common genotype. On the other hand, among the Turkish IV drug users

Table 3. Hepatitis C virus genotypes distrubution of autopsy cases with respect to nationality

		HCV Genotype								
Nationality	**Number (n)**	**1A (n,%)**	**1B (n,%)**	**1B/3 (n,%)**	**2 (n,%)**	**2/1A (n,%)**	**2/1B (n,%)**	**3 (n,%)**	**4 (n,%)**	**N/A (n,%)**
Turkey	18	8 (44.4)	1 (5.6)	1 (5.6)	-	-	-	4 (22.2)	-	4 (22.2)
Turkmenistan	4	-	2 (50)	-	-	-	-	2 (50)	-	-
Georgia	3	-	1 (33.3)	-	1 (33.3)	-	-	1 (33.3)	-	-
Pakistan	3	-	1 (33.3)	-	-	-	-	1 (33.3)	-	1 (33.3)
Syria	2	-	-	-	-	-	-	1 (50)	1 (50)	-
Uzbekistan	2	-	2 (100)	-	-	-	-	-	-	-
South Africa	1	-	-	-	-	-	-	1 (100)	-	-
Tanzania	1	1 (100)	-	-	-	-	-	-	-	-
Unknown	6	-	-	-	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	-
Total	40 (100)	9 (22.5)	7 (17.5)	1 (0.5)	2 (5)	1 (2.5)	2 (5)	11 (27.5)	2 (5)	5 (12.5)

HCV: Hepatitis C virus

genotype 1a (5/10; 50%) was found to be the most common genotype in our study. Of the all our autopsy cases cause of death was found to be related drug intoxication in 20 (50%) cases. For those reasons, much more prospective studies should be focused on people with HCV infection who are intravenous drug addicts.

There are various methods available for HCV genotyping. Techniques such as restriction fragment length polymorphism, allele-specific PCR and line probe assay are widely used to identify the HCV major and subtypes that are very common in North America, Europe and Japan. However, it has been stated that the standard reference and gold standard method comprise sequence analysis of HCV NS5, core, E1 and 5'-UTR regions and subsequent phylogenetic analysis (12). In our study, we used an automated PCR based method. Although the RT-PCR method has advantages such as being user independent, standard, automatic and yielding fast results, but in our study, the genotype of HCV subtypes could not be determined in 22.5% of cases. Like our study result, the genotype could not be determined in 9%, 25% and 27.3% of study population done in our country (13,14,33).

To our knowledge, this is the first HCV study with post-mortem cases in our country. More comprehensive molecular epidemiological studies are needed to understand the ways in which HCV enters our country and how it spreads. We think that import cases may affect the HCV biodiversity, and the detection of genotype 3 frequency in our country. Therefore, an effective HCV surveillance system should be established.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Commitee and Training and Scientific Research Commission of the Ministry of Justice Council of Forensic Medicine (approval number: 21589509/2019/125).

Informed Consent: It wasn't obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.Z., N.E., Design: N.Z., Ö.Y., M.N.A., Data Collection or Processing: N.Z., Ö.Y., Analysis or Interpretation: N.Z., N.E., Ö.Y., Literature Search: N.Z., N.E., Writing: N.Z., Ö.Y., N.E., M.N.A.

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Genotype Distribution of Hepatitis C Virus in Hatay Province of Turkey

Hatay İlinde Hepatit C Virüs Genotip Dağılımı, Türkiye

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ABSTRACT

Objectives: The treatment duration and response of chronic hepatitis C (CHC) are closely related to the genotypes of hepatitis C virus (HCV). This study aimed to determine the genotype distributions among CHC patients in the Hatay province of Turkey.

Materials and Methods: In this study, demographic data of 589 patients who received a therapy for CHC at the infectious diseases and gastroenterology clinics between June 2016 and May 2019 were retrieved from the hospital information system and medical charts of the patients and were retrospectively reviewed.

Results: The most common HCV genotype in our study was genotype 1b (66.9%), followed by genotype 2 (10.5%), genotype 1a (7.3%), genotype 4 (7.1%), genotype 3 (7%), and mixed genotype (1.2%). Six of the mixed genotypes were identified as 1b+4, while one was 1a+3. There was a statistically significant difference between females and males with regards to the HCV genotypes (p<0.001). Patients with genotype 1b tended to be older, while patients with genotypes 3 and 4 tended to be younger.

Conclusion: Genotype 1b is the most common HCV genotype in Hatay province, and it is followed by genotypes 2, 1a, 4 and 3. Compared to the studies conducted in previous years in Turkey, our study identified a lower rate for genotype 1b, along with an increase in the distribution rates of the other genotypes. Monitoring the changes in HCV genotype distribution is of vital importance to develop effective strategies in the treatment of HCV.

Keywords: Hepatitis C virus, genotype, Hatay

ÖZ

Amaç: Kronik Hepatit C (KHC) enfeksiyonunun tedavi süresi ve tedaviye verilen yanıt hepatit C virüs (HCV) genotipleri ile yakından ilişkilidir. Coğrafi bölgelere göre HCV genotiplerinin dağılımında farklılıklar vardır. Bu çalışmada Hatay ilindeki KHC hastalarında genotip dağılımlarının belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada KHC nedeni ile Haziran 2016-Mayıs 2019 tarihleri arasında enfeksiyon hastalıkları ve gastroenteroloji klinikleri tarafından tedavi başlanan 589 hastanın demografik verileri, hastane elektronik bilgi sistemi ve hasta dosyalarından retrospektif olarak incelendi.

Bulgular: HCV-RNA pozitif 589 hastada HCV genotiplerinin yüzdesi genotip 1b: %66,9, genotip 1a: %7,3, genotip 2: %10,5, genotip 3: 41 %7, genotip 4: %7,1 ve mix genotip: %1,2 olarak tespit edildi. Mix genotiplerin 6 tanesi 1b+4, bir tanesi 1a+3 olarak belirlenmiştir. Kadın ve erkek cinsleri arasında HCV genotipleri arasında istatistiksel olarak anlamlı farklılık görüldü (p<0,001). Genotip 1b hastaları daha ileri yaşlarda, genotip 3 ve genotip 4 hastalarının daha genç yaşlarda olduğu tespit edildi.

Sonuç: Hatay'da HCV genotip 1b en yaygın genotiptir ve bunu genotip 2, 1a, 4 ve 3 izlemektedir. Bizim çalışmamızda ülkemizdeki önceki yıllarda yapılan çalışmalara göre genotip 1b daha düşük tespit edilmiştir ve diğer genotiplerin dağılımında artış olduğu saptanmıştır. HCV genotip dağılımındaki değişikliklerin izlenmesi HCV'nin tedavisinde etkili stratejilerin geliştirilmesinde hayati öneme sahiptir.

Anahtar Kelimeler: Hepatit C virüs, Genotip, Hatay

Çabalak M, Bal T, Demir M, Ocak S, Önlen Y. Genotype Distribution of Hepatitis C Virus in Hatay Province of Turkey. Viral Hepat J. 2020;26:56-60.

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Introduction

Hepatitis C virus (HCV) causes both acute and chronic liver disease. It is estimated that there are 71 million people around the world that are infected with HCV. The disease becomes chronic and leads to the development of cirrhosis and liver cancer in a significant portion of patients. Approximately 399,000 people die every year due to hepatitis C-related cirrhosis and hepatocellular cancer (1).

Genome sequencing studies have identified seven genotypes and 67 subtypes of HCV (2). Methods used to identify the genotypes of HCV include DNA sequence analysis, type-specific polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism (RFLP), and the line probe assay, which is a commercial kit (3).

The distribution of HCV genotypes varies according to geographic regions. Genotypes 1 and 2 are the most common genotypes in the United States and Japan (4). Genotype 3 is the most common in Southeast Asia; genotype 4 is the most common in the Middle East, Egypt and Central Africa; genotype 5 is the most common in South Africa; and genotype 6 is the most common in Asia (5). Genotype 7 is found in Congo, Africa, while genotype 1b is reported to be the most common in Mediterranean countries (6). Studies in Turkey have shown that, similarly to the general distribution of genotypes worldwide, HCV genotype 1 is the most common genotype in the country (7,8).

Although pangenotypic therapies have been developed, the knowledge of the HCV genotype maintains its significance among the factors affecting the selection of the treatment regimen, the duration of treatment and the treatment success (9). This study aimed to determine the genotype distributions among patients with chronic HCV in the Hatay province of Turkey that is important in predicting the response to therapy.

Materials and Methods

This retrospective cross-sectional study retrospectively reviewed the data for 589 patients who were started on a therapy for chronic HCV between June 2016 and May 2019 by the Hatay Mustafa Kemal University Faculty of Medicine Healthcare Application Hospital, Clinic of Infections Diseases and Gastroenterology. The demographic data of the patients were retrieved from the hospital's electronic information system and the patients' files. Patients under the age of 18, patients with a co-infection with HBV or Human Immunodeficiency Virus, and foreign nationals were not included in the study.

For viral load determination, HCV-RNA levels were studied using real-time PCR method (COBAS AmpliPrep/COBAS Tagman, Roche Diagnostics, Germany), while the HCV genotypes were studied using the Real Time HCV Genotype II system (Anatolia geheworks, Turkey).

The study was performed with the approval of the Hatay Mustafa Kemal University Faculty of Medicine Ethics Committee (approval number: 09, date: 27.06.2019). Due to the retrospective design of the study informed consent was not obtained.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 21. The variables were investigated using histograms and Shapiro-Wilk test to determine whether or not they are normally distributed. The Mann-Whitney U test was used to compare the non-parametric variables. The chi-square test or Fisher's exact test, where appropriate, was used for categorical variables. A p-value of 0.05 or lower was considered to show a statistically significant result.

Results

The 589 patients who met the study inclusion criteria consisted of 286 (48.6%) males and 303 (51.4%) females with a median age of 64 years [interquartile range (IQR): 52-72 years]. The most common HCV genotype in our study was genotype 1b (66.9%; n=399), which was followed by genotype 2 (10.5%; n=62), genotype 1a (7.3%; n=43), genotype 4 (7.1%; n=42) and genotype 3 (7%; n=41) (Figure 1). Six of the mixed genotypes were identified as 1b+4, while one was identified as 1a+3. Genotypes 5 and 6 were not identified in our study.

A statistically significant difference was observed between female and male patients in terms of HCV genotype distribution ($p<0.001$). Patients with genotype 1b were predominantly female, with a rate of 58.4%, while cases infected with genotypes 2, 3 and 4 were predominantly male (59.7%, 90% and 88%, respectively).

The median age was 67 among cases with genotype 1b (IQR: 59-74), 60.5 among cases with genotype 2 (IQR: 34.2-75), 28 among cases with genotype 3 (IQR: 23-33), 34 among cases with genotype 4 (IQR: 30-45.5), 62 among cases with genotype 1a (IQR: 50-70), and 68 among cases with mixed genotype (IQR: 33-71). The mean age of patients infected with genotype 1 was higher than the mean age of the patients infected with other genotypes, and this difference was found to be statistically significant ($p<0.001$). Patients with genotype 3 and genotype 4 were younger ($p<0.001$) (Figure 2).

Discussion

The distribution of HCV genotypes varies considerably around the world. The most commonly observed genotype among adult patients with HCV worldwide is genotype 1 with a rate of 49%, and it is followed by genotype 3 (17.9%), genotype 4 (16.8%),

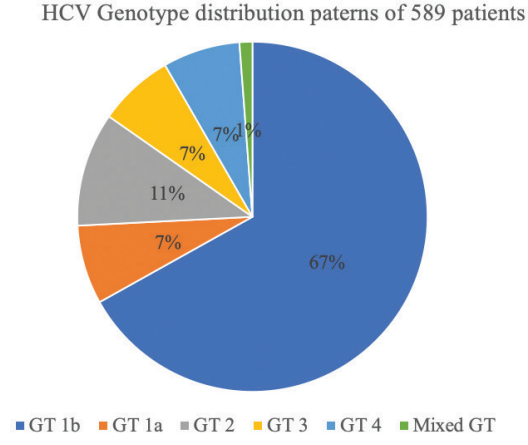

Figure 1. Distribution patterns of HCV genotypes
HCV: Hepatitis C virus, GT: Genotype

genotype 2 (11%), genotype 5 (2%) and genotype 6 (1.4%) in descending order (10).

Studies conducted in Turkey on the distribution of HCV genotypes have found that genotype 1 is responsible for approximately 90% of HCV infections, with the majority being genotype 1b. Other genotypes are observed less frequently. The present study identified genotype 1 as the most frequent genotype, which is similar to the situation in Turkey and in the world. The most frequently observed HCV genotype in our study was determined as genotype 1b (66.9%) Table 1.

In parallel with the data for Turkey, our study found high frequency rate for genotype 1b (66.9%). However, unlike other studies, our study determined that the distribution of genotype 1b has relatively decreased, while the distribution of other genotypes has increased. Our study also includes the highest number of patients from a single centre.

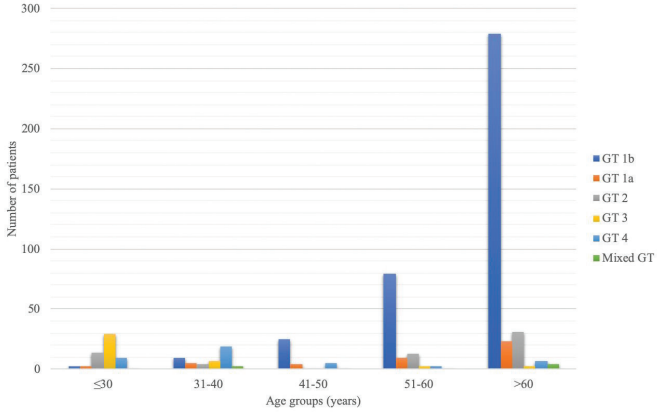

Figure 2. The genotype distribution according to age groups
GT: Genotype

The studies of Oztürk et al. (18) in Adana and Antakya, Borcak et al. (25) in Nevşehir and Akgün et al. (27) in Adıyaman found the distribution of genotype 2 to be higher than those reported in previously conducted studies in Turkey. The researchers attributed this to the increase in the use of intravenous drugs and the geographic location of these provinces. While we also identified an increase in the distribution of genotype 2 in our study, we did not associate this with the use of intravenous drugs.

A look at certain studies that have been published in Turkey in recent years shows that there is an increase in the frequency of genotype 3 (7,15,18,19,24,26). In a study performed by Sağlik et al. (24) in Antalya, the prevalence of genotype 3 was reported to be 11.1%, and 40% of these cases were foreign nationals. The researchers attributed the change in genotype distribution of HCV to the fact that Antalya is one of the most visited cities in the world and it has a high rate of immigration. In a study performed by Kirisci et al. (19) in Kahramanmaraş, the prevalence of genotype 3 was reported to be 40%, which is above the average values for Turkey. However, this observation may also be explained by the fact that the said study included a relatively small number of patients. Our study did not include foreign nationals, and 63.4% of patients with genotype 3 consisted of patients who used intravenous substances. This finding may also account for the fact that genotype 3 patients were generally younger.

Table 1. Distribution of hepatitis C virus genotypes observed in studies from different provinces or regions of Turkey

Study group	Number of patients	Year	Genotypes						Provinces/Regions
			1a	1b	1	2	3	4	
Ozacar et al. (11)	170	2001	10	81.2	-	2.4	0.6	1.2	İzmir
Bozdayi et al. (12)	365	2004	11	84	-	3	1	1	Ankara
Altuglu et al. (13)	345	2008	9.9	87.2	-	0.9	1.4	0.6	İzmir
Celik et al. (14)	178	2010	8.9	88.2	-	1.1	1.6	-	Sivas
Kucukoztas et al. (15)	115	2010	5.2	81.7	1.7	1.7	6.1	3.5	İstanbul
Gökahmetoğlu et al. (16)	146	2011	3.4	52.7	5.5	2.7	-	35.6	Kayseri
Kayman et al. (17)	375	2012	2.4	57.6	2.4	3.2	1.1	32	Kayseri
Oztürk et al. (18)	639	2013	1.9	71.2	-	11.9	13.3	1.7	Adana and Antakya
Kirisci et al. (19)	100	2013	-	-	60	-	40	-	Kahramanmaraş
Altuğlu et al. (20)	535	2013	12.9	80.4	0	1.5	3.7	1.5	İzmir
Tezcan et al. (21)	236	2013	1.7	84.7	5.9	2.1	4.2	0.8	Mersin
Buruk et al.(22)	304	2013	5.3	87.5	-	1.6	4.9	0.7	Trabzon
Aktaş et al. (23)	108	2014	8.3	87	-	-	3.7	1	Eastern Anatolia
Sağlik et al. (24)	422	2014	14.7	63.3	5.4	3.5	11.1	1.6	Antalya
Borcak et al. (25)	170	2014	-	37	45.1	14.5	1.2	0.6	Nevsehir
Zeytinli et al. (7)	554	2017	23.1	56.5	-	-	17.3	-	İstanbul
Özer Balin et al. (26)	71	2017	-	-	87.3%	2.8	9.9	-	Elazığ
Akgün et al. (27)	71	2018	8.4	71.8	4.2	11.27	4.2	-	Adıyaman
Karabulut et al. (8)	412	2018	38.8	37.4	6.3	4.6	10.7	2.2	İstanbul
Our study	589	2019	7,3	66.9	-	10.5	7	7.1	Hatay

Our study identified an increase in the distribution of genotype 4. Gökahmetoğlu et al. (16) and Kayman et al. (17) previously determined that the prevalence of HCV genotype 4 is higher in Kayseri compared to its prevalence in the rest of the country, but the researchers did not discuss possible reasons for this finding. While the rate of genotype 4 in our study was lower compared to that in Kayseri, it was still higher compared to the rates reported in other centres across Turkey. The authors believe that the findings on genotype distribution can be explained by geographic location.

Genotypes 5 and 6 were not detected in our study. In the studies by Tezcan et al. (21) and Çizmeci (28) in Turkey, only one person was found to have genotype 6.

A study carried out in Spain with 48,595 chronic HCV patients determined that genotypes 3 and genotype 4 patients are more common among men, while genotypes 1 and 2 patients are more common among women (29). Studies conducted in Western Europe, Russia and Israel have found similar results (30). In their study conducted in the Kahramanmaraş province of Turkey, Zeytinli et al. (7) observed no gender-related difference in genotype distribution, while in their study performed in Istanbul, Karabulut et al. (8) observed both age- and gender-related differences in genotype distribution, determining that genotypes 1 and 2 are more common among women, while genotype 3 and 4 are more common among men. In addition, they also observed genotype 1 being more frequent among elderly patients, and genotype 3 being more frequent among younger patients. In their study performed in Antalya, Sağlık et al. (24) observed that patients infected with genotype 1 are generally older than patients infected with the other genotypes; however, they identified no significant difference in terms of gender-related distribution among the patients infected with different genotypes. In our study, a significant difference was identified with regards to gender distribution between the patients infected with different genotypes, with genotypes 2, 3 and 4 being observed more commonly among males. It was found that genotype 1b is common among the elderly, and that most of the infected patients are women (Figure 3).

Study Limitations

The most important limitation of our study was the fact that all data were obtained from patients with CHC who directly presented to our hospital to receive effective antiviral therapy. Since our study did not include patients with unknown hepatitis C status and those who do not seek therapy, care should be taken while interpreting the results of the present study.

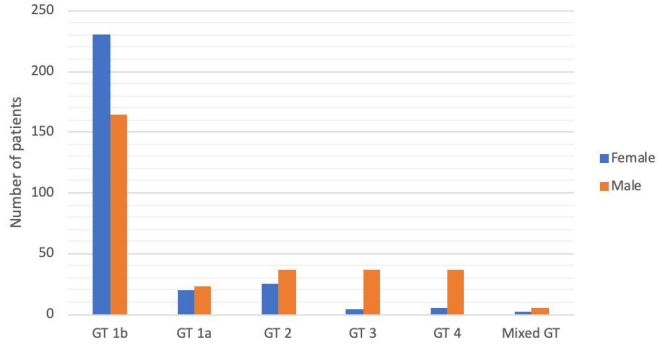

Figure 3. The genotype distribution according to gender
GT: Genotype

Conclusion

In our study, the most common HCV genotype in Hatay province was genotype 1b, which was followed by genotypes 2, 1a, 4 and 3 in terms of frequency. Compared to studies in Turkey that have been performed in previous years, we identified a lower frequency rate for genotype 1b, along with an increase in the distribution of the other genotypes. Monitoring the changes in the distribution of HCV genotype continues to be important for the selection of effective HCV therapies and for predicting treatment response. It is observed that there are regional differences in the genotype distribution in Turkey, which is why we believe it is important for each region to know its own epidemiological data.

Ethics

Ethics committee approval: The study was performed with the approval of the Hatay Mustafa Kemal University Faculty of Medicine Ethics Committee (approval number: 09, date: 27.06.2019).

Informed Consent: Due to the retrospective design of the study informed consent was not obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Ç., T.B., M.D., S.O., Y.Ö., Concept: M.Ç., T.B., Design: M.Ç., T.B., Data Collection or Processing: M.Ç., T.B., Analysis or Interpretation: M.Ç., T.B., M.D., S.O., Y.Ö., Literature Search: M.Ç., T.B., Writing: M.Ç., T.B.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declare that this study has not received any financial support.

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Research Article

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Intravenous Drug Use Rates and Results of Direct-acting Antiviral Treatment in Prisoner Patients

Mahkum Hastalarda İntravenöz İlaç Kullanım Oranları ve Direkt Etkili Antiviral Tedavi Sonuçları

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ABSTRACT

Objectives: Intravenous drug use (IVDU) is more common in prisoner patients, and this is a global problem. Hepatitis C virus (HCV) infection is higher in prisoners than general population. In our study, we aimed to examine the IVDU rates and direct-acting antiviral (DAA) treatment results of the prisoners who applied to Hatay Mustafa Kemal University Clinic of Infectious Diseases.

Materials and Methods: In our study, IVDU rates and HCV treatment results of 85 prisoners who applied to Hatay Mustafa Kemal University Faculty of Medicine Clinic of Infectious Diseases between January 2017 and December 2019 were retrospectively analyzed. Treatment results were evaluated by performing modified intention to tract (mITT) and per protocol (PP) efficacy analysis, respectively.

Results: The rate of IVDU was 37.7% in prisoners who were positive for HCV. Although sustained virological response (SVR) rate was 100% in PP analysis, SVR rate was determined as 80.5% in mITT analysis. Viral genotype 3 (41.6%) and genotype 4 (39%) were the most common.

Conclusion: However, data on HCV screening and treatment in prisons in Turkey is inadequate or too low. We think that with the use of DAAs, patients' compliance to treatment will increase, it is an important step for HCV eradication and multicenter studies should be conducted.

Keywords: Chronic hepatitis C, prisoners, direct acting antiviral, IV drug user

ÖZ

Amaç: Mahkum hastalarda intravenöz ilaç kullanımı (IVDU) sıklığı ve hepatit C virüs (HCV) enfeksiyonu prevelansı küresel olarak genel popülasyona göre daha yüksektir. Bu çalışmada Hatay Mustafa Kemal Üniversitesi Enfeksiyon Hastalıkları Kliniği'ne başvurup sağlık hizmeti alan mahkum hastalardaki IVDU oranlarının ve direkt etkili antiviral (DEA) tedavi sonuçlarının incelenmesi amaçlandı.

Gereç ve Yöntemler: Çalışmamızda Ocak 2017- Aralık 2019 yılları arasında Hatay Mustafa Kemal Üniversitesi Tıp Fakültesi Hastanesi Enfeksiyon Hastalıkları Kliniği'ne başvurup sağlık hizmeti alan toplam 85 mahkum hastanın IVDU oranları ve DEA tedavi sonuçları retrospektif olarak incelendi. Sırasıyla modifiye intention to tract (mITT) ve per protocol (PP) ile etkinlik analizi yapılarak tedavi sonuçları değerlendirildi.

Bulgular: HCV pozitif mahkum hastalarda intravenöz ilaç kullanım oranı %37,7 idi. PP analizinde kalıcı virolojik yanıt (KVY) %100 iken mITT analizinde bu oran %80,5 olarak saptandı. En sık viral genotip 3 (%41,6) ve genotip 4 (%39,0) saptandı.

Sonuç: Cezaevlerinde HCV taranması ve tedavisi açısından Türkiye'de yeterli veri yok veya çok azdır. DEA'ların kullanılması ile hastaların tedaviye uyumunun artacağını, HCV eradikasyonu için önemli bir adım olduğunu ve çok merkezli çalışmalar yapılması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: Kronik hepatit C, mahkum, direkt etkili antiviral, IV ilaç kullanımı

Çabalak M, Bal T. Intravenous Drug Use Rates and Results of Direct-acting Antiviral Treatment in Prisoner Patients. Viral Hepat J. 2020;26:61-64.

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Introduction

Hepatitis C virus (HCV) is a major global epidemic, and estimated 71 million people worldwide are chronically infected. Approximately 399,000 people die annually due to HCV-related liver failure and cancer in the world (1,2). In the developed countries, intravenous drug use (IVDU) is the main transmission route of HCV (2). In the literature, unsafe IVDU, sharing of drug paraphernalia, toothbrushes and shavers, tatooing have been identified as a risk factor for HCV infection transmission in prisoners (3,4,5,6). The prevalence of HCV in prisoners worldwide is up to 26%, and the incidence in prisoners who using intravenous drugs is up to 64% (3). Due to the physical conditions and psychological characteristics of prisoners, it is difficult for them to access and benefit from health services (7,8). Prisoners may have better access to health care and lower mortality rates in prisons than when they return to society (7,9). HCV treatment can be performed similar or better than the normal population In prisoners (10,11). All prisoners in prisons should be tested for HCV infection (12). HCV is now a preventable and treatable infection, but difficulties remain in reaching infected people (9,13). Prisons can provide a good opportunity to overcome these difficulties. Prison-based screening and treatment should be essential. However, data on HCV screening and treatment in prisons in Turkey is inadequate or too low. In our study, We aimed to discuss the treatment results and IVDU rates of prisoners who were followed up in our clinic due to HCV, by comparing them with other literature data in our country and in the world.

Materials and Methods

This retrospective, observational, single-center study was performed in prisoners who were followed up by Hatay Mustafa Kemal University Faculty of Medicine Hospital, Clinic of Infectious Diseases. Patients' ages, genders, demographic data, previous treatment experience, drug use, liver biopsy if available, viral load (HCV-RNA levels at 4th week of treatment and after the traetment, 12th and/or 24th week post-treatment) and viral genotype data were obtained from the hospital automation system and patient files retrospectively. Patients younger than 18 years old, who were coinfected with HBV and human immunodeficiency virus were not included in the study. The cases were treated with one of the direct-acting antiviral (DAA) drugs. The drugs used in the treatment are as follows; sofosbuvir ± ribavirin (SOF ± RBV), ombitasvir + paritaprevir/ritonavir (OBV + PTV/r) ± RBV, PrOD [(OBV + PTV/r) ± dasabuvir (DSV)] ± RBV, glekapravir + pibrentasvir and ledipasvir + SOF. RBV dose was determined according to the patient's weight. DAA drug selection and treatment decisions were made according to the Health Application Communique of the Turkish Social security institution guideline and the decision of physician responsible for treatment (14). HCV genotype and plasma HCV-RNA levels were determined by a real-time PCR assay, using either the COBAS AmpliPrep/COBAS Taqman (Roche Molecular Systems Inc., Pleasanton, CA, USA) or the Bosphore HCV Quantification Kit V2 (Anatolia Geneworks, Turkey) with a detection limit of 15 IU/mL and 25 IU/mL, respectively.

The primary outcome was the proportion of patients achieving a sustained virological response (SVR), which define as an undetectable HCV viral load at 12 weeks after completion of therapy. Effectiveness assessments other than SVR12 included: early virological response (EVR) (undetectable serum HCV-RNA at 4 weeks of therapy), virologic breakthrough (detectable HCV-RNA during treatment when previously undetectable) and relapse (detectable HCV-RNA after treatment when previously undetectable at the end of therapy.

The study was carried out with the approval of Hatay Mustafa Kemal University Faculty of Medicine Retrospective Ethics Committee (approval number: 10, date: 13.02.2020). Due to the retrospective design of the study informed consent was not obtained.

Statistical Analysis

Treatment efficacy analyzes were performed with both modified intention to tract (mITT) and per protocol (PP). PP analysis includes the level of HCV-RNA both post-treatment and after completing 12 weeks of follow-up. For mITT analysis, in addition to the HCV-RNA value measured prior to treatment, patients had to have a measured HCV-RNA value at least in the first month of treatment and all patients whose SVR12 was unknown were accepted as unresponsed when conducting mITT analysis. For statistical analysis, IBM SPSS version 23.0 statistical package program (SPSS Inc, Chicago, IL, USA) was used. The compatibility of variables to normal distribution was tested using the Kolmogrov-Smirnov test and histogram. Median and interquartile intervals were used for variables that do not fit the normal distribution.

Results

In our study, a total of 85 prisoner patients used DAA treatment between 2017-2019. Six of these patients were excluded from the study because they had never used the treatment and did not come to follow-up, and two patients were excluded from the study because their medication was just started. All of the patients were male. The rate of IVDU in HCV positive prisoners was 37.7% (29/77). To evaluate the effectiveness, mITT in 77 cases and PP analysis in 60 cases were used. While SVR was 100% in PP analysis, this rate was 80.5% in mITT analysis.

In our study, the average age of 77 patients who evaluated by mITT efficacy analysis was 30 [interquartile range (IQR): 25-33.5]. Eighteen cases (23.4%) in 2017, 21 cases (27.3%) in 2018, 38 cases (49.4%) in 2019 were included in our study. The number of prisoner patients whose treatment is started by years was shown in Figure 1.

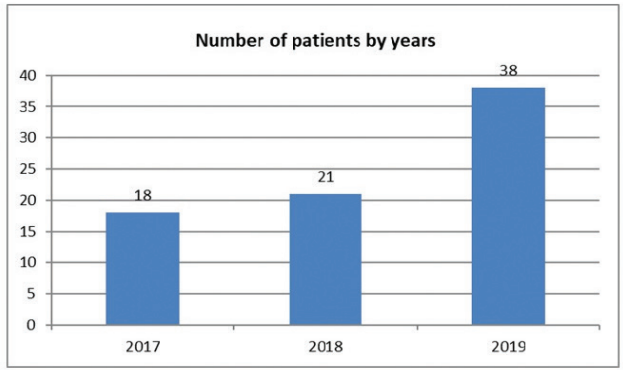

Figure 1. Number of patients by years

The genotype distribution of the patients is as follows; genotype 1a: 5 cases (13%), genotype 1b: 2 cases (2.6%), genotype 2: 7 cases (9.1%), genotype 3: 32 cases (41.6%), genotype 4: 30 cases (39.0%), mixed genotype: 1 case (1.3%). Viral genotype distribution in prisoner patients was shown in Figure 2. The treatments that patients receive are as follows; 22 patients SOF ± RBV (28.6%), 28 patients OBV + PTV/r (36.4%), 7 patients PrOD (OBV + PTV/r + DSV) ± RBV (9.1%), 19 patients glecapravir + pibrentasvir (24.7%), 1 patient SOF + LED (1.3%). RBV (71.4%) was used in 55 cases. No virological exacerbation and relapse were detected during treatment. EVR was obtained in 72 cases (93.5%). Only one case was treatment experienced.

Only 17 of 29 patients with a history of IVDU was achieved SVR. The rate of SVR in patients with IVDU was 17/29 (58.6%). There was a significant difference between with and without IVDU in terms of SVR (p=0.000). In our study, headache, bloating, weight loss and insomnia were observed as side effects in patients who were followed-up regularly. Especially in three patients using OBV + PTV/r + DSV ± RBV, minimal aspartate aminotransferase/alanine aminotransferase elevation and isolated bilirubin elevation were detected, but no treatment was discontinued due to serious adverse effects. In our study, liver biopsy was performed in six patients and no cirrhotic patient was detected.

Discussion

In studies evaluating the response of peg-interferon (IFN) + RBV therapy, SVR at the end of treatment was determined between 28% and 69% in prisoner patients with hepatitis C (1,15,16,17,18,19,20,21,22,23). In the study conducted by Ozger et al. (24), only 33 of the 99 patients who started Peg-IFN + RBV treatment had SVR at the 6th month after treatment. DAAs' used in HCV treatment are more effective, reliable and tolerable drugs compared to interferon-based regimens (25). In our study, SVR12 was obtained in 60 of 77 patients whose treatment was started. In patients who completed the treatment, SVR12 was 100%. Although they received DAA treatment, it was thought-provoking that SVR12 was not examined in 17 patients. Second-generation DAAs are a great improvement in the completion of the treatment and follow-up of prisoners, as their short course of treatment is reliable and tolerable (26). However, in our study, the most important reason for not continuing to treatment and follow-up was determined as the fact that prisoners did not come to follow-up after release. According to the study of Larney et al. (3), the prevalence of anti HCV in prisoners is 26%, while it can be up to 64% in IV drug addicted prisoners. In the same study, while anti HCV was 1.4% in the general population, anti HCV was found to be 16.4% in IV drug addicts (3). In our study, the rate of IVDU in prisoners with HCV infection was 37.6% (29/77). We think that this may be due to the low number of patients and geographical region differences. In the study conducted by Zampino et al. (25), The prevalence of anti HCV in convicted patients reported between 3% and 38% according to geographic region, IV drug use, age, duration of imprisonment, and prisoners' history. The most common genotypes in studies are genotype 1 and 3 (25,27). In Turkey, there are very few studies on prisoner patients. In the study conducted by Keten et al. (28), the most common genotype among prisoners in Turkey is genotype 3(68.1%). In the study of Ozger et al. (24), Genotype 3a is 66.7% (66/99). Unlike the literature, the remarkable result in our study was that genotype 4 was found to be 39% (30/77). However, we found the most common genotype is genotype 3, as 41.6% (32/77). We think that this difference may be due to geographical region difference. Only 17 of 29 patients with a history of IVDU reached SVR-12. SVR-12 was not known in 12 cases. The rate of SVR-12 in patients with a history of IVDU was 17/29 (58.6%). When patients with and without IVDU history were compared in terms of SVR, there was a significant difference. The antiviral treatment response in prisoners is similar to the general population (10,11). Unfortunately, treatment compliance is low in prisoners because treatment follow-up and management are difficult. In our study, it was found that treatment compliance was low, especially in patients with a history of IVDU. The most important reason for not continuing to treatment and follow-up was determined as the fact that prisoners did not come to follow-up after release. Side effects are an important factor affecting treatment results and continuation of the treatment, but in our study, no patient was discontinued their drugs due to drug-related side effects.

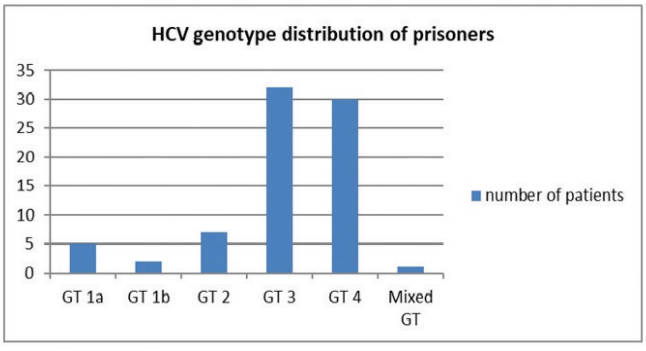

Figure 2. Viral genotype distribution in prisoner patients
HCV: Hepatitis C virüs, GT: Genotype

Study Limitations

The limitation of our study is that it is a retrospective study, the data is regional and the number of cases is low.

Conclusion

We think that prisoners provide a good opportunity to increase the diagnosis and treatment of HCV infection. We think that the compliance of patients to treatment will increase with the use of second-generation DAA drugs and it is an important step for HCV eradication and we suggest that multicenter studies should be conducted in our country.

Ethics

Ethics committee approval: The study was carried out with the approval of Hatay Mustafa Kemal University Faculty of Medicine Retrospective Ethics Committee (approval number: 10, date: 13.02.2020).

Informed Consent: Due to the retrospective design of the study informed consent was not obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.Ç., T.B., Design: M.Ç., T.B., Data Collection or Processing: M.Ç., Analysis or Interpretation: T.B., Literature Search: M.Ç., Writing: M.Ç.

Conflict of Interest: The authors declare no conflict of interest.

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Research Article

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Correlation between Hepatitis C Virus Antibodies in Saliva and Serum: A Safe Method for Epidemiological Studies

Tükrükten Ayrıştırılan Hepatit C Virüs Antikorları ile Serum HCV-RNA Arasındaki Korelasyonun Belirlenmesi

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ABSTRACT

Objectives: Hepatitis C virus (HCV) infection is an increasing public health problem in developing countries. A non-invasive method is required as blood sampling is an invasive method for detecting HCV antibodies. In this report, we examined the performance of a commercially available serological kit to detect HCV antibodies in saliva as a possible alternative to serum for epidemiological studies.
Materials and Methods: A total of 150 paired oral fluid and blood samples were collected from 75 anti-HCV-positive and 75 anti-HCV-negative individuals. Homemade swabs were used for saliva sampling instead of commercial products.
Modified Ortho HCV 3.0 SAVe ELISA kit was used to detect HCV antibodies in saliva, and blood samples were analyzed for anti-HCV and HCV-RNA.
Results: The sensitivity and specificity of this assay were 86.7% and 86.7% in saliva. Out of 38 participants who were positive for HCV-RNA in serum, 36 were also positive for HCV antibodies in saliva.
Conclusion: The implementation of a non-invasive method such as saliva collection is easy, economical, and can be done by unskilled personnel. According to our sensitivity and specificity results, the modified ELISA method for anti-HCV detection in saliva with the use of a different saliva collection system can be an alternative technique for epidemiological surveys.
Keywords: Hepatitis C virus, epidemiology, antibody, Saliva

ÖZ

Amaç: Tükrükte hepatit C virüsüne (HCV) karşı bulunan antikorlar ile kandaki HCV-RNA ve antikorları arasındaki ilişkiyi araştırmak ve epidemiyolojik çalışmalar ön planda olmak üzere saha çalışmalarında bu yöntemin kullanılabilirliğini saptamaktır.
Gereç ve Yöntemler: Polikliniğimize başvuran 75 anti-HCV (+) ve 75 anti-HCV (-) olgunun tükrük ve kan örnekleri alındı. Tükrük almak için çeşitli ticari kitler yerine hastaların ağız bakımı için geliştirilmiş olan sünger çubuklar kullanıldı. Alınan örnekler modifiye Ortho HCV 3.0 SAVe ELISA (Ortho Clinical Diagnostics, US) kiti ile çalışıldı. Kan örnekleri ise uygun koşullarda alınıp anti-HCV ve HCV-RNA tetkikleri çalışılmak üzere hastanemiz merkez laboratuvarına gönderildi.
Bulgular: Çalışılan 75 anti-HCV (+) hastanın otuzsekizinde (%50,7) HCV-RNA (+), 65'inde (%86,7) tükrük anti-HCV (+) saptandı. Kontrol grubundaki 75 olgudan 10'unun (%13,3) tükrüğünde anti-HCV (+) saptandı. Serum anti-HCV altın standart olarak kabul edildiğinde tükürük anti-HCV duyarlılığı %86,7 ve seçiciliği %86,7 olarak sonuçlandı. HCV-RNA (+) olan 38 hastanın 36'sında (%94,7) tükrük anti-HCV (+) bulundu.
Sonuç: Anti-HCV'nin tükrükte araştırılması oldukça yeni bir tanı yöntemi olup tükrük örneğinin alınması kolay, acısız, hızlı ve daha az teknik ekipman gerektiren bir yöntemdir. Non-invaziv bir teknik olması, bulaş riski oluşturmaması, eğitimli personele ihtiyaç duyulmadan kişinin kendi kendine örnek almasına olanak tanıması bu tekniği saha çalışmaları için çekici kılmaktadır. Çalışmamızın duyarlılık ve seçicilik sonuçlarına göre bu yöntem epidemiyolojik araştırmalarda kullanılmaya aday alternatif bir tekniktir. Ülkemizde bu konu ile ilgili yapılan araştırma olmaması, ayrıca hazır tükrük alma kiti kullanılmadan yapılan ilk araştırma olması nedeniyle de ayrı bir yeri bulunmaktadır.
Anahtar Kelimeler: Hepatit C virüsü, epidemiyoloji, antikor, tükrük

Eren Kutsoylu OÖ, Kuruüzüm Z, Yüce A. Correlation between Hepatitis C Virus Antibodies in Saliva and Serum: A Safe Method for Epidemiological Studies. Viral Hepat J. 2020;26:65-68.

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Introduction

Hepatitis C virus (HCV) infection is an important public health problem, with an estimated 170 million people infected worldwide (1). Although available data are limited, it is estimated prevalence of HCV is higher in developing than developed countries (2). Common risk factors for HCV infection are blood transfusion and intravenous drug use, and sexual and vertical transmission (3). HCV infection primarily affects liver and can cause cirrhosis and hepatocellular carcinoma later in life.

Although sensitive and specific serologic tests are available, difficulty in obtaining blood samples and risk of disease transmission to health personnel through needlestick injury are disadvantages that limit their acceptance outside a clinical setting. Therefore, a safer, non-invasive alternative to blood sampling is required. Collection of saliva is easy, non-invasive, painless and safe to carry out. In epidemiological studies, especially those involving screening large populations, saliva sampling is quicker than blood sampling and there is no need for specialized staff (4).

Several saliva collection methods have been developed, e.g. Omni-SAL (Saliva Diagnostic Systems, Inc., Vancouver, WA, USA), Orapette (Trinity Biotech, Dublin, Ireland), OraSure (Epitope Technologies, Inc., Bethlehem, PA, USA), and Salivette (Sarsted Ltd., Leicester UK). The study reports use of an in-house saliva collection device, which is easy to produce and offers a more affordable method for clinical and epidemiology studies conducted in developing countries.

Materials and Methods

Study group

The study group consisted anti-HCV seropositive and anti-HCV seronegative patients (n=75 for both groups). HCV-seropositive patients were those undergoing follow-up visits at the Infectious diseases and clinical microbiology outpatient clinic. HCV-seronegative participants were those who attended the same outpatient clinic for other reasons. The study was approved by the Institutional Ethical Comittee Dokuz Eylül University (approval number: 200895). Prior informed consent was obtained from all participating patients, who were >18 years of age.

Sample collection

Each participant donated two blood samples (16 mL) and one saliva sample (6 mL), collected at the same time. Serum from one sample was stored at -20 °C and the other at -80 °C until used. Saliva samples were collected using sterile foam swabs (3 cmX 1.5 cmX 5.5 cm). Patient was requested to hold the foam swab in mouth for two minutes, then swab was placed in one ml aliquot of Universal Transport Medium (Copan Italia S.p.a., Brescia, Italy), centrifuged at 3.000 g for 15 minutes and supernatant stored at -20 °C until used.

Laboratory assays

Serum samples were tested for anti-HCV antibodies using an Architect i2000 SR kit (Abbott Laboratories, Ltd. Saint-Laurent, Québec, Canada). Anti-HCV-positive serum samples were then tested for presence of HCV-RNA using Artus HCV-RG-RT-PCR assay kit (QIAGEN Gmbh, Hilden, Germany).

Saliva samples were tested for anti-HCV antibodies using Ortho HCV 3.0 SAVe ELISA kit (Ortho Diagnostics, Amersham, UK) with a modified protocol to increase sensitivity (5). In brief, 110 µl aliquot of saliva solutions and control samples were incubated for 16-20 hours at ambient temperature (15-30 °C) with shaking in a 96-well microtiter plate. Then plate was washed with buffer (supplied by the manufacturer), 200 µl aliquot of horseradish peroxidase-conjugated murine anti-human immunoglobulin G (IgG) monoclonal antibodies was added to each well, and plate was incubated for another three hours at ambient temperature, washed as described above before addition to each well of 200 µl of substrate and incubation for 30 minutes at room temperature in the dark. Reaction was terminated with 50 µl of 4 M sulfuric acid solution and $A_{490\ nm}$ measured with a microplate spectrophotometer (Multiskan FC Microplate Photometer; Thermo Fisher Scientific Oy, Oulu, FINLAND). Final $A_{490\ nm}$ values determined by a receiver operating characteristic (ROC) curve analysis.

Statistical Analysis

Detection of anti-HCV antibodies in serum samples was used as a gold standard for the assessment of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Ortho HCV 3.0 SAVe ELISA assay (Ortho Diagnostics) in saliva. Cut-off value was calculated according to ROC curve analysis as value with highest specificity and the sensitivity. Values ≥0.133 were considered positive and values <0.133 considered negative.

Results

Mean age of the participants was 57±12.5 years in the test group and 58±17 years in control group, with 47% males. Nine participants in the test group were in a hemodialysis program. All participants had negative Human Immunodeficiency Virus serology.

Difference between mean $A_{490\ nm}$ value (1.32±1.023 and 0.17±0.24) of anti-HCV antibody ELISA in saliva samples of test and control group respectively is statistically sigificant (Mann-Whitney U test; mwu: 27, p-value <0.001), as was in serum samples (data not shown). Sensitivity, specificity, NPV and PPV value of saliva samples employing the modified Ortho HCV 3.0 SAVe ELISA (Ortho Diagnostics) was 87% for each parameter (Table 1).

In the HCV-seropositive group, 38 (51%) patients were HCV-RNA positive, of whom 36 (95%) were positive for anti-HCV antibodies in saliva. Of the 37 patients who were HCV-RNA negative, 29 (78%) had anti-HCV antibodies in saliva. Similar relationship was observed between serum HCV-RNA positivity

Table 1. Anti-HCV antibody-positive and -negative samples from participants at the infectious diseases and clinical microbiology outpatient clinic of a university hospital in İzmir, Turkey

Anti-HCV antibody* Positive		Serum anti-HCV		Total
		Negative		
Saliva	Positive	65	10	75
	Negative	10	65	75

*Determined using Ortho HCV 3.0 SAVe ELISA kit (Ortho Diagnostics, Amersham, UK).
HCV: Hepatitis C virus

and $A_{490\ nm}$ values in saliva. Difference between mean $A_{490\ nm}$ value (1.65±0.94 and 0.66±0.78) in saliva of test patients with positive and negative serum HCV-RNA respectively is also statiscally significant (Mann-Whitney U test; mwu: 277.5, p-value <0.001). Higher mean $A_{490\ nm}$ value was observed in serum HCV-RNA positive than negative group.

Discussion

Saliva sampling is an easier and less invasive method compared to serum sampling. Previous reports indicate that saliva is an appropriate specimen diagnosis of different infectious diseases, in particular viral infections that pose a hazardous risk to health personnel in the situation of needle prick accidents (5). In addition, there are various devices for collecting saliva as well as commercial tests geared for assaying saliva samples (Table 2).

In our study, an in-house saliva collecting device consisting of a sterile foam placed in a subject's mouth was employed, a low-cost alternative to more expensive commercial saliva collecting devices. Using a saliva-dedicated commercial ELISA kit, there was good concordance between paired serum and saliva samples in detecting anti-HCV antibodies. Sensitivity and specificity of this assay for saliva samples were high (>85%).

In comparison with serum, Ig levels are 800-1000 folds lower in oral fluid, where IgA is predominant (6). Lee et al. (7) evaluated the use of an HCV antibody rapid test device with venous blood, fingerstick blood, serum, plasma, and oral fluid. They observed a slightly lower sensitivity (98.1%) with oral fluid, which they attributed to conditions of oral health, use of oral care products, and consumption of food and drink (7). Cha et al. (8) used the HCV antibody rapid test device with oral fluid and reported a clinical sensitivity was 97.8%. This may be responsible for low sensitivity of HCV antibody detection in saliva in the event of low anti-HCV antibody titers and negative HCV-RNA levels (9,10). It is worth noting a patient negative for saliva anti-HCV antibody was HCV-RNA positive and had previously been diagnosed as rheumatoid arthritis and treated with immunosuppressive agent.

Using saliva for antibody detection brings out new approaches which makes anti-HCV detection easier. Self-saliva collection is one these advantages. User friendly and easy to apply test is cost effective, convenient and time saving compared to complex laboratory test methods which requires highly skilled and experienced laboratory specialist and more accessible in underserved communities and isolated populations (11).

Cost-effectiveness also makes saliva sampling attractive. In a cost-effectiveness study rapid antibody saliva testing costs was determined much more lower when compared to testing via venipuncture (10).

Table 2. Saliva anti-HCV antibody detection employing different saliva collection devices and ELISA methods

Reference	Saliva collection device	Anti-HCV antibody assay	Positive anti-HCV antibody number	Negative anti-HCV antibody number	Percent sensitivity (95% CI)	Percent specificity (95% CI)
Elsana et al. (6)	No device	Abbott HCV 2.0[a]	73	52	90	100
Bello et al. (9)	Salivette	Abbott HCV 3.0[a]	152	108	94 (89-97)	99 (94-100)
Van Doornum et al. (10)	Salivette	Abbott HCV 3.0 SAVe ELISA[a]	102	50	77.5	98
	Salivette	Monolisa[b]	102	50	79.4	98
	Omni-sal	O. HCV 3.0 SAVe ELISA[c]	102	50	76.5	98
	Omni-sal	Monolisa[b]	102	50	76.5	94
Judd et al. (11)	Orasure	O. HCV 3.0 SAVe ELISA[c]	253	392	92 (87-95)	99 (98-100)
	Salivette	HCV 3.0 SAVe ELISA[c]	252	389	74 (62-79)	99 (97-100)
Lucidarme et al. (12)	Salivette	Monolisa[b]	45	63	78	99
De Cock et al. (13)	Oracol	O. HCV 3.0 SAVe ELISA[c]	73	73	89 (79-95)	100 (94-100)
Amado et al. (5)	Orasure	United Biomedical HCV 4.0d	16	89	75 (47-92)	98 (92-99)
Gonzalez et al. (4)	Orasure	O. HCV 3.0 SAVe ELISA[c]	45	45	87 (72-94)	100 (90-100)
Moorthy et al. (14)	Omni-sal	Hepanostika HCV Ultrad	49	93	81.5	92.5
Present study	In-house device	O. HCV 3.0 SAVe ELISA[c]	75	75	87	87

[a]Abbott Laboratories, Chicago, IL, USA, [b]Sanofi, Diagnostic Pasteur, Marnes-la-Coquette, France, [c]Ortho Diagnostics, Amersham, UK, [d]UBI Diagnostics, Beijing, China. HCV: Hepatitis C Virus, CI: confidence interval

Study Limitations

Discrepancies in sensitivity and specificity determined in other studies (Table 2) may be related to collection devices used, study populations and saliva ELISA method employed. Sensitivity and specificity of saliva anti-HCV antibody ELISA was lower in our study compared to literature data, likely related to use of a different method for saliva collection. A comparative study using the collection device of the present study and at least one of the commercially available collection devices should be able to provide an answer.

Conclusion

A combination of the new saliva collection method with a modified commercial ELISA assay yielded acceptable results. Sensitivity and specificity indicate that this method should be suitable for epidemiological surveys, obviating risk to health personnel using invasive procedure and more acceptable by infants and young children.

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Ethics

Ethics Committee Approval: The study was approved by the Institutional Ethical Comittee Dokuz Eylül University (approval number: 200895).

Informed Consent: Prior informed consent was obtained from all participating patients, who were >18 years of age.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - O.Ö.E.K., Concept: O.Ö.E.K., A.Y., Design: O.Ö.E.K., Z.K., Data Collection or Processing: O.Ö.E.K., Analysis: O.Ö.E.K., Literature Search: O.Ö.E.K., Writing: O.Ö.E.K.

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Research Article

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The Effect of Switch Treatment on Liver Fibrosis and qHBsAg Levels in Patients with Chronic Hepatitis B

Kronik Hepatit B Hastalarında Switch Tedavisinin Karaciğer Fibrozu ve qHBsAg Düzeylerine Etkisi

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ABSTRACT

Objectives: The aim of this study was to evaluate the relationship between clinical, biochemical, serological parameters, fibroscan imaging in terms of fibrosis and quantitative hepatitis B surface antigen (qHBsAg) levels in patients with chronic hepatitis B (CHB) infection whose treatment has been switched from LAM to TDF.

Materials and Methods: The study included 19 patients with CHB and under the LAM treatment. The gender, age, comorbidity, medications, routine laboratuary, creatinine clearance, bone mineral density, transient elastography for stage of liver fibrosis and qHBsAg level were examined.

Results: Ten of 19 patients were female and 9 were male. When the qHBsAg titers of the patients at 6th and 12th months were compared, there was a statistically significant decrease in qHBsAg titers of the patients after the 12th month. There was a significant decrease in liver fibrosis measurements at the 12th month of treatment change. There was a statistically significant positive correlation between qHBsAg titers and fibroscan values at baseline and 12th month.

ÖZ

Amaç: Bu çalışmanın amacı, tedavisi LAM'den TDF'ye geçen kronik hepatiti B (KHB) enfeksiyonu olan hastalarda klinik, biyokimyasal, serolojik parametreler, fibroskan görüntüleme ile fibrozis ve kantitatif hepatit B yüzey antijeni (qHBsAg) düzeyleri arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntemler: Çalışmaya LAM tedavisi altındak 19 KHB hastası dahil edildi. Cinsiyet, yaş, yandaş hastalıkları, ilaçlar, rutin laboratuvar tetkikleri, kreatinin klirensi, kemik mineral dansitesi, fibrozis derecelendirmesi için transient elastografi ölçümü ve qHBsAg seviyeleri belirlendi.

Bulgular: 19 hastanın 10'u kadın 9'u erkekti. Hastaların 6. ve 12. ayındaki qHbsaq titreleri değerlendirildiğinde, 12. ayın sonunda qHBsAg titrelerin anlamlı ölçüde düşüş saptandı.

Karaciğer fibrozis ölçümlerinde de tedavi değişikliğinin 12. ayında anlamlı düşüş saptandı. Başlangıçtaki ve 12. aydaki qHBsAg titreleri ile fibroscan ölçümleri arasında istatistiki olarak anlamlı bir pozitif korelasyon saptandı.

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This study is generated from the first author's thesis study.

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Conclusion: In this study, the replacement of LAM with TDF may prevent the resistance problem, and also the decrease in fibrosis values and/or qHBsAg levels may contribute to the prevention of HCC and cirrhosis have been showed.
Keywords: Lamivudine, tenofovir, switch treatment, liver fibrosis, hepatitis B

Sonuç: Bu çalışmada LAM yerine TDF'ye geçilmesi ile direnç probleminin çözülebileceği, aynı zamanda qHBsAg titrelerinde ve fibrozis değerlerinde düşüş sağlanarak HCC ve sirozdan da korunulabileceği gösterilmeye çalışılmıştır.
Anahtar Kelimeler: Lamivudin, tenofovir, tedavi değişikliği, karaciğer fibrozis, hepatit B

Introduction

Chronic hepatitis B (CHB) virus infection is the most common cause of cirrhosis, end stage liver disease, hepatocellular carcinoma (HCC) and death from liver disease in Turkey. Since long term suppression of HBV replication with antivirals is associated with histological improvement, the main goal of therapy for (CHB) is to suppress HBV replication in a sustained fashion and thereby to prevent progression to cirrhosis and development of HCC. Currently, there are 2 classes of drugs approved for the treatment of CHB: pegylated interferon alfa and nucleot(s)ide analogues, i.e. lamivudine, adefovir, entecavir, tenofovir and telbivudine.

Lamivudine is the first nucleoside analogue for the treatment of CHB. Lamivudine has been shown to be effective in patients with hepatitis B e antigen [HBeAg (+)] and HBeAg (-) chronic HBV infection whether they had compensated and decompensated cirrhosis (1,2). In treatment naive patients with HBeAg (+) CHB, HBeAg seroconversion rates were shown to be 16-18% at year 1 (3).

In HBeAg (-) CHB, undetectable HBV-DNA levels were achieved in 60-70% of patients at year 1; however, HBV DNA became positive in 90% of patients after stopping therapy (4,5). Lamivudine was also shown to prevent disease progression, HCC development, and the need for liver transplantation in compensated and decompensated cirrhosis (6,7,8). However, resistance to lamivudine develops in 11-24% of patients with HBeAg (+) CHB and 6-18% of those with HBeAg (-) CHB, and reaches up to 70% of patients after 8-year treatment (9).

Tenofovir is a potent nucleotide analogue with high genetic barrier to resistance (10). It is effective in both treatment naive and lamivudine/entecavir resistant chronic HBV infection (11). In HBeAg (+) CHB, tenofovir achieved undetectable HBV-DNA levels in 76%, HbeAg seroconversion in 21%, hepatitis B surface antigen (HbsAg) seroconversion in 3%, alanine aminotransferase (ALT) normalization in 68% and histological improvement in 74% of patients after 1-year therapy. In HBeAg (-) CHB, tenofovir achieved undetectable HBV-DNA levels in 93%, ALT normalization in 76% and histological improvement in 72% of patients after 1-year therapy (12). According to recently published EASL and AASLD guidelines, tenofovir is one of the first-line therapies for CHB (1,13).

In the present study, we aimed to evaluate the efficacy of tenofovir in chronic hepatitis B patients in whom serum HBV-DNA had become negative on lamivudine therapy and it was switched to tenofovir in the absence of lamivudine resistance with respect to biochemical and serological responses. We also aimed to evaluate the efficacy of tenofovir on liver fibrosis by transient elastography and associations of these variables with quantitative HBsAg (qHBsAg) levels.

Materials and Methods

The study included 19 CHB patients who had been followed up at gastroenterology outpatient clinic. Patients were screened for serum HBV-DNA, [aspartate aminotransferase (AST), ALT, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT)], albumin, bilurubin, prothrombin time and creatinin levels. Of them, patients who were on lamivudine therapy and had undetectable serum HBV-DNA were included. 3 cc blood samples were obtained from patients for the measurement of serum qHBsAg.

After centrifuged, serum samples were stored in deep freeze at -80 °C. At 6th and 12th months of therapy, same procedures were repeated. At the end of study period, serum qHBsAg levels were measured using Abbott Architect i2000sr device and Abbott ArchitectHBsAg 6C36 quantitative kit which was based on chemiluminescent microparticle immonoassay. Serum qHBsAg concentration ≥0.05 IU/mL was considered positive. When qHBsAg was > 250.00 IU/mL, serum samples were 1/500 diluted.

Anti-HBc, anti-HBs, HBeAg, and anti-HBe were measured using AbbottArchitect i2000sr device. Serum HBV-DNA was measured using Rotor-Gene Q, Qiagen device and Artus HBV QS-RGQ kit by real time polymerase chain reaction (PCR).

Serum and urine biochemistry tests were measured using Abbott/Architect C16000 device. Twenty four-hour urinary protein was measured by turbidimetric assay using benzethonium chloride as denaturating agent. Liver fibrosis was evaluated by transient elastography using CAP featured Fibroscan 502 Touch (SNF60121)-Probe xl (SN90226)-c2.0.0.0 at the start and 12th month of TDF therapy.

The liver stiffness measurement results were expressed in 1.5-75 kPa and CAP measurement results were expressed in 100-400 dB/m. Liver stiffness measurement was performed by one physician and factors that might hamper the liver stiffness were taken into account. More than 10 successful acquisitions, success rate >60% and IQR/M rate <30% were considered reliable.

Lamivudin was switched to TDF 245 mg qd. Written informed concent was obtained form the all patients. Local Ethic Committee approval was taken from the Kocaeli University with the approval number of 21.91016-2016/15.2.

Statistical Analysis

Statistical analyses were made using SPSS (SPSS, Inc, Chicago, IL, USA) for Windows 17.0. For the evaluation of the study data, in addition to descriptive statistical methods (mean ± standard deviation), the Student's t-test and the Mann-Whitney U test were used to establish potential differences between the averages of two independent groups for parameters with and without normal

distributions, respectively. One-Way ANOVA test and Friedman test were used for the comparison of dependent quantitative variables. Correlation analysis between quantitative variables were performed using Pearson correlation test. For comparisons of qualitative data, the chi-squared test was used. The results in the 95% confidence interval and p values <0.05 were considered to be significant.

Results

The study included 19 patients. All patients were Caucasian, and 10 of them were (52.6%) were female with a mean age of 54.7±9.6 years. Eight patients (42.1%) were smoker and none of them were alcohol drinker. Body mass index was 25.0-29.9 kg/m^2 in 9 patients (47.4%), 30.0-39.9 kg/m^2 in 2 patients (9.5%), and ≥40.0 kg/m^2 in 3 patients (6.3%). None of them had family history of hepatocellular carcinoma. HBeAg was negative in all patients.

Liver stiffness measurements at the beginning and 12th month of therapy were 7.4±3.8 kPa and 6.2±2.9 kPa, respectively (p=0.013). Decreasing in liver stiffness measurement was 0.95 (0.30-2.37) kPa. Liver stiffness measurement was decreased in 17 patients (89.4%) and increased in 2 patients (10.6%).

At the beginning of therapy, 11 patients had osteopenia (57.9%) and 8 patients had normal bone mineral density (43.1%). At the 12th month of therapy, 12 patients had osteopenia (47.4%) and 10 patients had normal bone mineral density (52.6%). The difference in bone mineral density between the beginning and the 12th month of therapy was not statistically significant (p=0.500).

There was not statistically significant difference in qHBsAg levels between the beginning and the 6th month of therapy (p=0.114). However, qHBsAg levels showed a significantly (p=0.003) decreasing as 9.1±18.5% at the 12th month of therapy while comparing with the beginning.

There was statistically significant correlation between decrease in qHBsAg levels and improvement in liver stiffness measurement (Table 1).

Discussion

Lamivudin has been used in the treatment of chronic HBV infection; however, resistance is an important problem due to low genetic barrier. Although it effectively suppress HBV replication in the short term, virological breaktrough and flare can occur due to mutations.

Therefore, lamivudin is no longer recommended as a first line therapy in the treatment of chronic HBV infection (14,15,16).

The frequency of resistance to oral antivirals increases as the time goes by. After 5 years of therapy, resistance to lamivudin and adefovir reaches to 70% and 29%, respectively.

Table 1. Correlation between qHBsAg and liver stiffness measurement

		Fibroscan 1	Fibroscan 2
HBsAg 0	r	0.511	0.502
	p	0.030	0.028
HBsAg 12	r	0.492	0.476
	p	0.038	0.039

HbsAg: Hepatitis B surface antigen

Resistance to telbivudin was reported as 22% after 2 years. On the other hand, resistance to entecavir is only about 1.2% and resistance to tenofovir has not been reported yet (13,17).

Besides effective and maintained suppression HBV replication, another goal of therapy is prevention of side effects. Because of low antiviral activity and low genetic barrier to resistance, lamivudin is no longer recommended as a first line therapy in the treatment of chronic HBV infection (18).

In a study by Marcellin et al. (19) 5-year TDF therapy resulted in histologic improvement in 87% patients and regression of fibrosis in 51% of patients. Only 9 of 641 patients developed side effect which had led to discontinuation of therapy (19). In the present study, histological improvement rate ocurred in 17 of 19 (89.7%) of patients.

Routine follow up with liver biopsy is not recommended to monitör histological improvement due to its invasiveness and complications. Moreover, repeat liver biopsy result does not lead to therapy modification. Therefore, noninvasive tests are used to evaluate the efficacy of antivirals on histological activity. Of them, fibroscan is increasingly used to evaluate liver fibrosis.

In meta-analysis of 50 studies, the area under the receiver operating characteristic (ROC) curves of fibroscan to predict significant fibrosis (F2), advanced fibrosis (F3) and cirrhosis (F4) were 0,84 (95% CI, 0.82-0.86), 0.89 (95% CI, 0.88-0.91) and 0.94 (95% CI, 0.93-0.95), respectively. As a result, transient elastography seems excellent in the prediction of cirrhosis and successfull in the prediction of advanced fibrosis, while there is variation in the prediction of significant fibrosis according to etiology of liver disease (20).

In an Asian metaanalysis, the area under the ROC curves of fibroscan to predict significant fibrosis (F2), advanced fibrosis (F3) and cirrhosis (F4) in chronic hepatitis B patients were 0.859 (95% CI, 0.857-0.860), 0.887 (95% CI, 0.886-0.887) and 0.929 (95% CI, 0.928-0.929), respectively (21). In another metaanalysis, Tsochatzis et al. (22) evaluated the diagnostic value of transient elastopgraphy. In this study, threshold liver stiffness measurement for F2, F3 and F4 fibrosis were 7, 9.5 and 12 kPa, respectively. Transient elastography had good sensitivity [0,83 (95% CI 0.79-0.86)] and specifity [0.89 (95% CI 0.87-0.91)] in the prediction of cirrhosis; however, they concluded that it should be used with caution in thew prediction different fibrosis stages in daily practice because there was not approved threshold values (22). In a cross sectionel study, the thresholds in the prediction of F2, F3 and F4 fibrosis were 6,9, 7,9 and 9,6 kPa, respectively (23). In the present study, liver fibrosis was improved significantly after 1 year therapy with TDF. Although the duration of treatment was short, the improvement in liver fibrosis is important and seems a valuable clue in the choise of antiviral agent.

HBsAg quantification has not been established in treatment monitorization yet. It was shown that there was correlation between cccDNA and intrahepatic HBV-DNA, and qHBsAg (24). In the present study, serum HBV DNA levels remained undetectable throughout therapy.

Moreover, qHBsAg levels decreased significantly after 1 year of TDF therapy. In a study, Pfefferkorn et al showed that qHBsAg level was reliable marker to establish inactive HBV carrier state (25). Tan et al. (26) showed that qHBsAg was not a reliable marker to

differentiate HBeAg negative chronic hepatitis B and inactive HBV carrier state. Sali et al. (27) also found similar results. On the other hand, there are studies who showed opposite findings (28,29). In the present study, decrease in qHBsAg levels supports TDF has favorable effects on cccDNA and intrahepatic HBV despite small number of patients.

Study Limitations

The most important limitations of our study are the small number of patients and the patients were evaluated without biopsy.

Conclusion

Potents antivirals as TDF should take place of lamivudin in the treatment of chronic hepatitis B. Significant decreasing in liver stiffness measurement and qHBsAg levels at long term can prevent the development of cirrhosis and hepatocellular carcinoma. There is need for studies made with large patient groups and in long term following up.

Ethics

Ethics Committee Approval: Local Ethic Committee approval was taken from the Kocaeli University Faculty of Medicine (approval number: 21.91016-2016/15.2).

Informed Consent: Verbal and written informed consent received.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., F.G., Concept: M.S., G.Ş., Design: M.S., G.Ş., Data Collection or Processing: S.B.A., G.D., Analysis and/or Interpretation: S.B.A., M.K., Literature Search: E.Y., H.S., Writing: E.Y., M.K.

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Research Article

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Seroprevalence of Hepatitis A in Children Followed-up with the Diagnosis of Chronic Hepatitis B Infection

Kronik Hepatit B Enfeksiyonu Nedeniyle Takip Edilen Çocuklarda Hepatit A Seroprevalansı

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ABSTRACT

Objectives: To investigate the seroprevalence of hepatitis A virus (HAV) infection in children with chronic hepatitis B virus (HBV) infection and to immunize patients who did not have anti-HAV immunoglobulin (Ig) G level in protective titer.

Materials and Methods: A retrospective analysis was made in 79 children with chronic HBV infection between January 2017 and December 2018.

Results: Of the patients with a mean age of 11.5±4.6 years (1-18 years), 64.6% were boys and 35.4% were girls (male/female=51/28). Anti-HAV (Ig) G was positive in 72.2% (n=57) of the cases. According to gender, the rate of sero-positivity was found to be 72.5% (n=37) in boys and 71.4% (n=20) in girls. HAV immunization was determined at the lowest rate (8.1%) in the 14-18 years age group. Although 3 patients vaccinated against HAV were anti HAV-IgG negative, and 39 patients who were not vaccinated were anti-HAV IgG positive.

Conclusion: The seroprevalence of HAV should also be evaluated in children with chronic HBV infection. Morbidity and mortality due to HAV infection can be prevented by vaccinating non-immune patients.

Keywords: Chronic hepatitis B infection, hepatitis A virus, super infection, seroprevalence, child

ÖZ

Amaç: Kronik hepatit B virüs (HBV) enfeksiyonu olan çocuklardaki hepatit A virüs (HAV) enfeksiyonu seroprevalansının araştırılması ve koruyucu titrede anti-HAV immünoglobulin (Ig) G düzeyine sahip olmayan hastaları bağışıklama amaçlanmıştır.

Gereç ve Yöntemler: Ocak 2017 ile Aralık 2018 tarihleri arasında kronik HBV enfeksiyonu tanısı ile izlenen 79 çocuk hastanın tıbbi kayıtları geriye dönük olarak incelendi.

Bulgular: Yaş ortalaması 11,5±4,6 yıl (1-18 yıl) olan hastaların %64,6'sı erkek, %35,4'ü kız idi (Erkek/Kadın=51/28). Olguların %72,2'sinde (n=57) anti-HAV immünoglobulin (Ig) G pozitif bulundu. Cinsiyete göre, sero-pozitiflik oranının erkeklerde %72,5 (n=37), kızlarda ise %71,4 (n=20) oranlarında olduğu belirlenmiştir. On dört-18 yaş grubu olgularda HAV bağışıklamasının en düşük oranda (%8,1) olduğu tespit edilmiştir. HAV'ye karşı aşılanmış 3 hastada anti HAV-IgG negatif olmasına karşın, aşılanmamış 39 hastada anti-HAV IgG pozitif bulundu.

Sonuç: Kronik HBV enfeksiyonu olan çocuklarda HAV seroprevalansının da değerlendirilmesi gerekir. Bağışık olmayan hastaların aşılanması ile HAV enfeksiyonuna bağlı morbidite ve mortalite önlenebilir.

Anahtar Kelimeler: Kronik hepatit B enfeksiyonu, hepatit A virüsü, süper enfeksiyon, seroprevalans, çocuk

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Introduction

Although hepatitis A virus (HAV) infection is widespread throughout the world, in developing countries in particular it continues to be a signifcant public health problem (1). HAV leads to different clinical tables ranging from asymptomatic infection to fulminant hepatitis (2). HAV infection may not always show a classic course and acute liver failure may be seen in approximately 1% of cases. Acute liver failure is seen more often when there is an underlying liver disease or chronic hepatitis B infection (3).

Chronic hepatitis B virus (HBV) infection in children can be confused with acute viral hepatitis associated with HAV super infection. Compared with healthy children, HAV super-infection in patients with chronic HBV infection can lead to higher morbidity and mortality (4). Fulminant liver failure may be seen more often in these cases (5). Therefore, children followed up with a diagnosis of chronic HBV infection should be evaluated in respect of immunity to HAV infection and children who are not immune should be vaccinated (6).

The aim of this study was to investigate the seroprevalence of HAV in children being followed up with a diagnosis of chronic HBV infection and to immunise patients who did not have an anti-HAV immunoglobulin (Ig)G level at a protective titer. It was also planned to screen family members of cases with chronic HBV infection and vaccinate those who were not immune to HBV.

Materials and Methods

Approval for this retrospective cohort study was granted by the Non-Interventional Research Ethics Committee of Fırat University (approval number:13/14, date:19.07.2018). A retrospective examination was made of the medical records of 79 paediatric patients who were followed up with a diagnosis of chronic HBV infection in the Paediatric Gastroenterology Department of Şanlıurfa Training and Research Hospital between January 2017 and December 2018. The clinical and laboratory data obtained were recorded on forms prepared for the study. The patients were separated into 3 age groups as 1-6 years old, 7-13 years old, and 14-18 years old.

Statistical Analysis

Data obtained in the study were analysed statistically using IBM-SPSS vn. 22 software. Variables were stated as mean ± standard deviation values, or number and percentage (%). The chi-square test was used in analyses. A value of $p<0.05$ was accepted as statistically significant.

Results

The cases included in the study all had hepatitis B surface antigen (HBsAg) positivity for longer than 6 months. The whole patient group of 79 children comprised 51 (64.6%) boys and 28 (35.4%) girls with a mean age of 11.5±4.6 years (range, 1-18 years). Of these cases, 16 (20.3%) were in the 1-6 years age group, 26 (32.9%) were in the 7-13 years age group and 37 (46.8%) were in the 14-18 years age group. The median HBsAg level was determined as 1022 IU/mL (Table 1).

Table 1. Distribution of the cases by age and gender

	n (%)
Gender	
Female	28 (35.4)
Male	51 (64.6)
Age	
1-6 years	16 (20.3)
7-13 years	26 (32.9)
14-18 years	37 (46.8)

Anti-HAV IgG positivity was determined in 72.2% (n=57) of all the cases. According to gender, the anti-HAV IgG seroprevalence was found to be 72.5% (n=37) in boys and 71.4% (n=20) in girls. The hepatitis A immune status of the cases according to age is shown in Table 2. As the 1-6 years age group coincided with the introduction of the hepatitis A routine vaccination program in October 2012, 15 (93.8%) of the 16 cases in that group had been vaccinated. The 39 (49.33%) patients not vaccinated against HAV were thought to have had subclinical or asymptomatic HAV infection.

When comorbidities were examined, 2 (2.5%) cases with chronic HBV infection were found to have hepatoblastoma (these patients had undergone liver transplantation), 1 (1.3%) of which had cerebral palsy, and the other 1 (1.3%) had thalassemia major. Anti-HAV IgG positivity was determined in both of these patients. Lamivudine was being used by 4 patients because of chronic HBV infection, tenofovir by 2 patients and adefovir by one patient. Three of these patients were determined with anti-HAV IgG positivity. The 4 cases without immunity to HAV infection were administered Hepatitis A vaccination.

Discussion

As a result of improved socio-economic and hygiene conditions in Turkey in recent years and the inclusion of hepatitis A vaccination into the routine vaccination program, acute viral HAV infection has significantly decreased. Consequently, HAV infection has shifted to adolescents and adults (7).

Table 2. Hepatit A seroprevalence according to the age groups

Age group	Hepatit A seroprevalence		
	Not vaccinated (n, %)	Vaccinated (n, %)	Total (n, %)
1-6 years	1 (6.3)	15 (93.8)	16 (20)
7-13 years	23 (88.5)	3 (11.5)	26 (33)
14-18 years	34 (91.9)	3 (8.1)	37 (47)
$\chi 2$=46, 472, p=0.0001			

HBV infection continues to be a significant public health problem throughout the world in general (8). Chronic HBV infection is seen in approximately 400 million people (5% of the global population) (9). Hepatitis B vaccination was included in the national vaccination program in Turkey in 1998. Although a reduction has been seen in the seroprevalence of chronic HBV infection with the implementation of HBV vaccinations, this infection is still encountered in children (10).

HAV infection generally has a mild clinical course but in those with chronic HBV infection, it may show a worse course (11). Therefore, children and adolescents with chronic HBV infection should be evaluated in respect of immunity to HAV infection, and those without immunity should be vaccinated (9).

The majority of the cases in this study followed up for a diagnosis of chronic HBV infection were seen to be children or adolescents in the 14-18 years age group, who were born before the inclusion of hepatitis B vaccination in the national vaccination program. This finding can be accepted as a sign of the success of routine hepatitis B vaccination in Turkey. There has also been seen to be a significant reduction in chronic HBV infection in children as a result of increased awareness of HBV infection together with the implementation of vaccinations (10).

Anti-HAV IgG positivity was determined in 72.2% of the cases in this study followed up with a diagnosis of chronic HBV infection. Of the cases determined with anti-HAV IgG positivity, 15 had been included in the national vaccination program and 6 had been vaccinated for hepatitis A by their family. The 39 cases that had not been vaccinated were thought to have had subclinical or asymptomatic HAV infection.

When evaluation was made according to age groups, anti-HAV IgG positivity was seen most (75%) in the 1-6 years age group. Of the 4 cases in this age group with anti-HAV IgG negativity, 1 had not been vaccinated, and the other 3 had not formed anti-HAV IgG positivity at a protective titer despite vacination. In the 7-13 years age group, anti-HAV IgG positivity was determined in 53.8%. Three of the children in this age group had been vaccinated by their families and the other 11 children were thought to have had subclinical or asymptomatic HAV infection. In the 14-18 years age group, 83.8% were determined to have immunity to HAV. In this age group, 3 children had been vaccinated by their families and the other 28 children were thought to have had subclinical or asymptomatic HAV infection. The course of HAV infection in childhood is generally benign. In more than 70% of cases the clinical course is asymptomatic, but occasionally it can progress to liver failure (12). In the current study, anti-HAV IgG positivity was seen to increase together with age, which was consistent with the findings of other studies (7).

In a previous study in Turkey, conducted in Ankara, HAV seroprevalence in patients diagnosed with chronic HBV infection was reported to be 34% in the group aged <20 years (13). In another multicentre study in Turkey, positive hepatitis A seroprevalence was determined at the level of 73.8% in patients <19 years old who were followed up for chronic hepatitis B infection (14). A study in Konya reported hepatitis A seroprevalence as 28% in cases followed up for chronic HBV infection and aged <20 years (15). Together with these findings, the higher hepatitis A seroprevalence in the current study was thought to be due to differences between regions. It could also be attributed to the fact that our hospital is located in a region of Şanlıurfa which has been recently settled, has a large number of refugees, a low socio-economic level and insufficient infrastructure.

Shavakhi et al. (16) determined positive hepatitis A seroprevalence in 71.4% of Iranan cases aged 10 -20 years who developed chronic liver disease because of viral or autoimmune hepatitis and Wilson's disease. In a study in Korea by Kim et al. (17), positive hepatitis A seroprevalence was determined at the rate of 22.2% in cases aged <20 years who were followed up for chronic HBV infection, and it was stated that hepatitis A seroprevalence increased with age. In another study in Korea, Lee et al. (18) reported zero positive hepatitis A seroprevalence in cases aged 11-20 years who were followed up with a diagnosis of chronic hepatitis B. The seroprevalence of hepatitis A has been reported at the levels of 9.8% in the 21-30 years age group, 46.3% in the 31-40 years age group and 94.9% in cases aged >40 years. Although complications are not seen in the course of HAV infection in the majority of cases, there are cases that have developed acute liver failure. Accordingly, children with chronic HBV infection are at a higher risk than the normal population of developing acute liver failure associated withh HAV super infection.

Study Limitations

The limitations of our study were its retrospective nature and short study period.

Conclusion

Children diagnosed with chronic hepatitis B should be evaluated in respect of immunity to HAV infection. With vaccinations of those without immunity, the morbidity and mortality associated with HAV super infection, which can be seen in these patients, can be prevented. In respect of community health, the family members of patients diagnosed with chronic HBV infection should be evaluated in respect of HBV infection, and those without immunity should be vaccinated.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Fırat University Faculty of Medicine (approval number: 13/14, date: 19/07/2018).

Informed Consent: Informed consent was not obtained from the parents of the patients in this study because of the retrospective nature of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Data Collection and/or Processing: U.D., U.A., Analysis and/or Interpretation: U.D., U.A., Literature Search: U.A., U.D., Writing Manuscript: U.D.

Conflict of Interests: The authors have no conflict of interests to declare.

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Research Article

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Diagnostic Performance of Non-invasive Fibrosis Indexes in Hepatitis B Related Fibrosis

Hepatit B İlişkili Fibrosiste Non-invaziv Fibrozis Göstergelerinin Tanısal Performansı

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ABSTRACT

Objectives: The aim of this study was to evaluate the diagnostic performance of non-invasive fibrosis markers [AST to platelet ratio (APRI), Fibrosis Index based on four factors (FIB-4) İndex, AST/platelet/GGT/Alpha-fetoprotein (AFP) Index (APGA), FI, Fibro-quotient (FibroQ), AST/ALT ratio (AAR), GGT/Platelet ratio (GPR), Platelet-age-phosphatase-AFP-AST (PAPAS) and S-Index] in chronic hepatitis b (CHB) patients.

Materials and Methods: Treatment naive CHB patients who underwent liver biopsy were screened. Four hundred seventeen patients were included in the study. Fibrosis stage was reevaluated according to ISHAK score. The diagnostic efficacy of non-invasive fibrosis indicators for significant fibrosis (≥F3) and cirrhosis (≥F5) was evaluated. The diagnostic performance of the non-invasive markers was defined as the AUROC value of ≥0.9 as excellent, 0.9> AUROC ≥0.8 as good, 0.8> AUROC ≥0.7 as moderate and AUROC <0.7 as poor.

Results: AUROC values of S-index, GPR, APRI, FIB-4 index, FibroQ and PAPAS for diagnosing significant fibrosis were 0.683, 0.667, 0.679, 0.679, 0.585, 0.606 respectively. AUROC values of S-Index, GPR, APGA and FIB-4 index, APRI, FibroQ, PAPAS, FI for diagnosing cirrhosis were 0.841, 0.833, 0.819, 0.802, 0.767, 0.700, 0.697, 0.620 respectively.

Conclusion: Diagnostic performance of S-Index for diagnosing cirrhosis and significant fibrosis was found superior to other indexes, but diagnostic performance of all these indexes was poor in predicting significant fibrosis. Diagnostic performance of S-Index, APGA, GPR, and FIB-4 index were good in determining cirrhosis.

Keywords: Hepatitis B, Liver fibrosis, non-invasive fibrosis indexes.

ÖZ

Amaç: Bu çalışmada kronik hepatit B (KHB) hastalarında non-invaziv fibrozis göstergelerinin [AST/trombosit oranı (APRI), dört faktöre dayalı fibrozis İndeksi (FIB-4), AST/platelet/GGT/alfa-fetoprotein (AFP) indeks (APGA), FI, fibro-quotient (FibroQ), AST / ALT oranı (AAR), GGT/trombosit oranı (GPR), Trombosit-Yaş-fosfataz-AFP-AST (PAPAS) ve S-index] tanısal performanslarının değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Karaciğer biyopsisi yapılan tedavi naiv KHB tanılı hastalar tarandı. Çalışmaya 417 hasta dahil edildi. Fibrozis evreleri ISHAK skoruna göre tekrar değerlendirildi. Non-invaziv göstergelerin anlamlı fibrozis (≥F3) ve siroz (≥F5) için diagnostik etkinliği değerlendirildi. Non-invaziv göstergelerin tanısal performansı AUROC değeri ≥0,9 ise mükemmel, 0,9> AUROC ≥0,8 ise iyi, 0,8> AUROC ≥0,7 ise orta ve AUROC <0,7 ise zayıf olarak tanımlandı.

Bulgular: S-index, GPR, APRI, FIB-4 Index, FibroQ and PAPAS skorlarının anlamlı fibrozis için AUROC değerleri sırası ile 0,683, 0,667, 0,679, 0,679, 0,585, 0,606 idi. Siroz tanısı için S-index, GPR, APGA and FIB-4 index, APRI, FibroQ, PAPAS, FI skorlarının AUROC değerleri sırası ile 0,841, 0,833, 0,819, 0,802, 0,767, 0,700, 0,697, 0,620 idi.

Sonuç: Siroz ve anlamlı fibrozis tanısı için S-indeksin tanısal performansı diğer göstergelerden üstün saptandı, fakat tüm göstergelerin anlamlı fibrozisi ön görmedeki tanısal performansları zayıf idi. S-index, APGA, GPR ve FIB-4 indeksin sirozu belirlemedeki tanısal performasları iyi idi.

Anahtar Kelimeler: Hepatit B, karaciğer fibrozisi, non-invaziv fibrosis göstergeleri

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Introduction

Hepatitis B virus (HBV) infection is one of the major causes of morbidity and mortality around the world. About 240 million people worldwide are known to have chronic HBV infection (1). The prevalence of chronic HBV infection in adult population in Turkey is 4%, also 40-45% of all patients with chronic hepatitis and cirrhosis have HBV infection (2).

To reduce the mortality associated with cirrhosis and hepatocellular carcinoma caused by HBV infection, it is important to start treatment in optimal time. Liver fibrosis grade is the most important indicator for timing of treatment. Also, it is a predictor of treatment response and prognosis (3,4,5). Liver biopsy is the gold standard for evaluating fibrosis. However, liver biopsy is an invasive procedure and it is not always accepted by patients and requires expert histopathological interpretation. There are also limitations of biopsy such as interobserver variability and sample variability (6). These drawbacks have led to conduction of studies on the evaluation of liver fibrosis by non-invasive methods. According to research in this field, it may be possible to diagnose fibrosis grade with using fibrosis biomarkers.

Direct fibrosis biomarkers (enzymatic indicators, collagen markers, glycoproteins and matrix-metalloproteinase indicators and glycosaminoglycans) reflect fibrogenic changes and extracellular matrix cycle at the cellular level in the liver. However, these indicators are not liver-specific, but also have disadvantages such as cost and availability difficulties in routine clinical practice (7). Indirect markers include gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time, albumin and bilirubin levels, reflecting alteration in hepatic function. These markers are also useful in diagnosing, evaluating severity and assessing the prognosis of liver diseases (7).

Combination of different indirect fibrosis markers such as AST to platelet ratio (APRI), Fibrosis Index based on four factors (FIB-4), AST/platelet/GGT/Alpha-fetoprotein (AFP) Index (APGA), Fibrosis Index, Fibro-quotient (FibroQ), AST/ALT ratio (AAR), GGT/Platelet ratio (GPR), Platelet-age-phosphatase-AFP-AST (PAPAS) and S-index can improve sensitivity and specificity of these tests (7,8,9,10,11,12,13,14,15,16).

However, most of these scores have not been validated in independent data sets, therefore they cannot be used routinely in clinical practice (17). The aim of this study was to evaluate the diagnostic performance of APRI, FIB-4 Index, APGA, FI, FibroQ, AAR, GPR, PAPAS and S-index in chronic hepatitis B (CHB) patients.

Materials and Methods

A total of 466 consecutive treatment naive CHB patients who underwent liver biopsy between 2012 and 2017 were screened. Demographic, serologic and biochemical data performed within one month before the biopsy were recorded from file and computer database of patients. CHB defined as hepatitis B surface antigen positivity for more than six months.

Patients who have hepatitis C, delta virus, human immunodeficiency virus (HIV) infection, with a history of alcohol intake higher than 20 gr/day, accompanying autoimmune hepatitis, fewer than 6 portal areas on liver biopsy, and lack of any biochemical parameters used to calculate non-invasive markers were excluded.

Non-invasive fibrosis scores (APRI, FIB-4, APGA, FibroQ, FI, AAR, GPR, PAPAS, S-index) of patients were calculated. Methods for calculating non-invasive markers are shown in Table 1.

Liver biopsies of all patients were reevaluated by an experienced pathologist who was blinded to the clinical and laboratory findings. Fibrosis stage and histological activity were recorded according to ISHAK score. The diagnostic efficacy of non-invasive fibrosis indicators for significant fibrosis (≥F3) and cirrhosis (≥F5) was evaluated.

The diagnostic performance of the non-invasive markers was defined as the AUROC value of ≥0.9 as excellent, 0.9> AUROC ≥0.8 as good, 0.8> AUROC ≥0.7 as moderate and AUROC <0.7 as poor (18). This study was approved by the Local Ethical Committee of Ümraniye Training and Research Hospital (approval number: B10.1TKH.4.34.H.GP.0.01/34). İnformed consent of patients couldn't obtained due to retrospective design of study.

Statistical Analysis

Descriptive (mean, standard deviation, minimum, median, maximum) statistics were used to define continuous variables. The relationship between independent two categorical variables was evaluated by Fisher's exact test. The comparison of two

Table 1. Calculation methods of noninvasive fibrosis markers

APGA: Log (Index) = 1.441+0.1490 Log (GGT)+0, 3308.log (AST)-0, 5846.log (PLT)+0.1148 log (AFP+1)
FIB-4: Age (year) × AST ÷ PLT (10^3/L) × √ALT
FI: 8.0-0.01 × PLT (10^3/L) - Albumın (g/dL)
FibroQ: [10 x age x AST × INR] ÷ [PLT (10^3/L) × ALT]
S-index: 1000 × GGT (IU/L) ÷ [PLT (10^9/L) × Albumın^2 (g/dL)]
APRI: [AST/(ULN*) ÷ plt (10^3/L)] × 100
AAR: AST ÷ ALT
PAPAS: [Log (Index + 1) = 0.0255 + 0.0031 × age + 0.1483 × log (ALP) - 0.004 × log (AST) + 0.0908 × log (AFP + 1) - 0.028 × log (pLT 10^3/L]
Gpr: [GGT (IU/L)/(ULN**)] / [pLT (10^3/L)] × 100

APRI: Aspartate transaminase to-Platelet Ratio Index, FIB-4: Fibrosis Index based on four factors, AAR: Aspartate transaminase to- Alanin transaminase ratio, APGA Index: AST/Platelet/GGT/Alpha-fetoprotein Index, FibroQ: Fibro-quotient, PAPAS: Platelet-Age-phosphatase-alfa fetöprotein - aspartate transaminase, GPR: GGT Gama-glutamil transferaz to platelet ratio, FI: Fibrosis Index, ALP: Alkaline phosphatase, AST: Aspartat aminotransferaz, ALT: Alanin aminotransferaz, PLT: Platelet, ULN: Upper limits of normal, GGT: Gama glutamil transferase, AFP: Alfafeto protein, INR: İnternational normalized ratio, *ULN of ALT: 40 IU/mL, **ULN of GGT: 63 IU/mL

continuous variables who distributed not normally was evaluated by Mann-Whitney U test. Logistic regression analysis was performed to identify independent risk factors for significant fibrosis and cirrhosis. Diagnostic performance of non-invasive fibrozis markers were evaluated by receiver operating curve (ROC) analysis. Significance was set at a p-value of <0.05. The analyzes were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

Results

Forty-nine patients who have at least one of the exclusion criteria were excluded from the study. Four hundred seventeen patients were included in the study. Flow chart the of study is shown in Figure 1.

1. Demographic Characteristics of Patients

One hundred and sixty-one (38.6%) of the patients were female and 256 (61.4%) were male. The mean age was 42.26±11.88 years. Two hundred and twenty-one (52.7%) of the patients had significant fibrosis, 80 (19.1%) had advanced fibrosis and 29 (6.9%) had cirrhosis. Demographic characteristics of the patients are shown in Table 2.

2. Risk Factors Associated with Fibrosis

2.1 Factors associated with significant fibrosis

Risk factors for significant fibrosis were determined as AST [p=0.014, odds ratio (OR): 1.026, 95% confidence interval (CI) Lower: 1.005-95% CI Upper: 1.048], GGT (p=0.001, OR: 1.022, 95% CI Lower: 1.008-95% CI Upper: 1.035), Albumin (p=0.009, OR: 0.456, 95% CI Lower: 0.252-95% CI Upper: 0.825) and PLT levels (p=0.001, OR: 0.994, 95% CI Lower: 0.990-95% CI Upper: 0.997). These findings are shown in Table 3.

2.2 Factors Associated with Cirrhosis

Risk factors for cirrhosis (F≥5) was determined as male gender (p=0.020, OR: 4.078, 95% CI Lower: 1.246-95% CI Upper: 13.348), GGT (p=0.031, OR: 1.013, 95% CI Lower: 1.001-95% CI Upper: 1.025) and AFP level (p=0.006, OR: 1.062, 95% CI Lower: 1.017-95% CI Upper: 1.109). These findings are shown in Table 3.

3. Diagnostic Performance of Non-invasive Markers

3.1 Significant fibrosis

Statistically significant difference was found between F≥3 and F <3 groups in terms of APGA, FIB-4 Index, FibroQ, S-index, APRI, PAPAS, GPR Index distributions (Mann-Whitney U, p<0.05). The mean values of these markers were higher in the patients with significant fibrosis. In the ROC analysis, S-index, GPR, APRI, FIB-4 Index, FibroQ and PAPAS scores showed poor diagnostic performance (AUROC <0.7). AUROC value S-index, GPR, APRI, FIB-4 index, FibroQ and PAPAS for diagnosing significant fibrosis were 0.683, 0.667, 0.679, 0.679, 0.585, 0.606 respectively. Cut off points, sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), positive and negative likely ratios (LR) of these markers in diagnosing significant fibrosis are shown in Table 4. ROC analysis of non-invasive markers was shown in Figure 2. FI, APGA Index and AAR were not useful in the diagnosis of significant fibrosis.

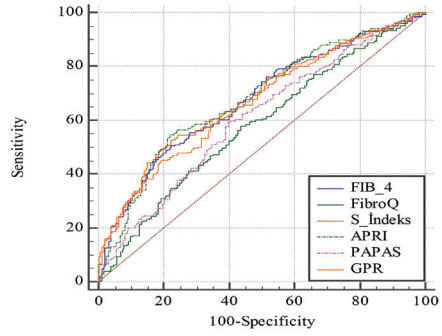

Figure 2. ROC analysis of non-invasive markers in prediction of significant fibrosis

FIB-4: Fibrosis Index based on four factors, FibroQ: Fibro-quotient, APRI: Aspartate transaminase to-Platelet Ratio Index, PAPAS: Platelet-Age-phosphatase-alfa fetöprotein - aspartate transaminase, GPR: GGT Gama-glutamil transferaz to platelet ratio, , ROC: Receiver operating characteristic

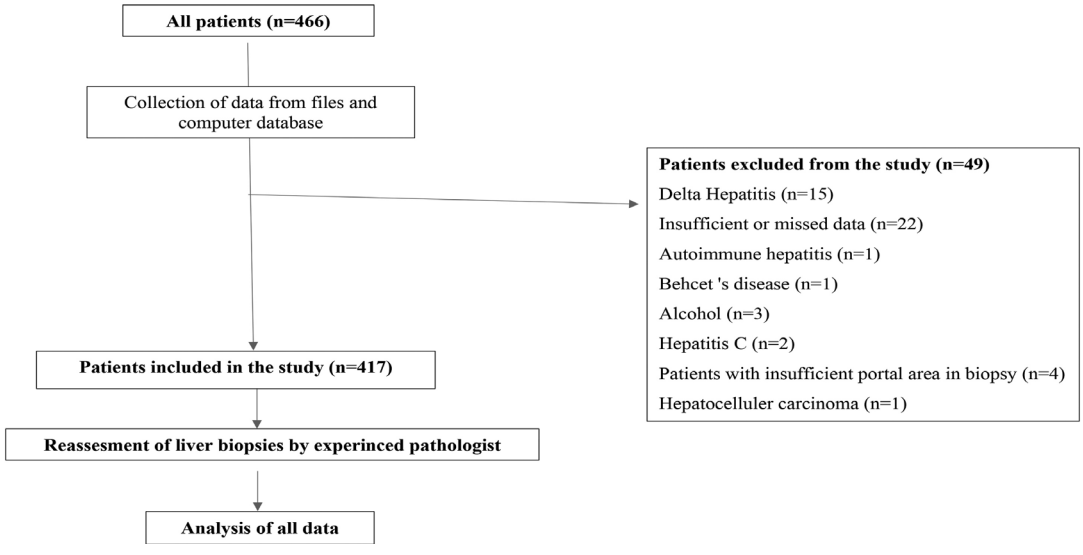

Figure 1. Flow chart the of the study

Table 2. Demographic and baseline characteristics of the patients (n=417)		
Parameters	**(n=417, (%) Mean ± SD**	**Median (min-max)**
Age	42.26±11.88	42 (18-73)
Sex		
Male	-	256 (61.4%)
Female	-	161 (38.6%)
	Mean ± SD	**Median (min-max)**
ALT (IU/L)	64.68±87.6	39 (6-862)
AST (IU/L)	43.04±49.55	30 (12-686)
GGT (IU/L)	32.72±27.95	24 (7-250)
ALP (IU/L)	78.17±24.17	74 (27-210)
Total Bilirubin (mg/dL)	0.87±1.29	0.7 (0.2-25)
Albumin (g/dL)	4.24±0.45	4.3 (0.2-7.8)
INR	1.07±0.11	1.06 (0.83-1.69)
AF (ng/mL)	4.11±6.32	2.85 (0,81-84.52)
PLT (10^3/L)	218.17±59.21	215 (76-550)
HBV DNA (IU/mL)	1.5±7.1 x107	1.2 $x10^5$ (3.1$x10^1$-9.2$x10^9$)
Liver Fibrosis (ISHAK)		
≥F2	-	387 (92.4%)
≥F3 (Significant fibrosis)	-	221 (52.7%)
≥F4 (Advance fibrosis)	-	80 (19.1%)
≥F5 (Cirrhosis)	-	29 (6.9%)
Anti-HBe positive	-	342 (82.5%)
NASH + CHB	-	15 (3.6%)
NAFLD + CHB	-	97 (23.3%)

ALP: Alkaline phosphatase, AST: Aspartat aminotransferase, ALT: Alanin aminotransferase, PLT: Platelet count, ULN: Upper limits of normal, GGT: Gama glutamil transferase, AFP: Alfafeto protein, INR: International normalized ratio, NAFLD: Non-alcholic fatty liver disease, NASH: Non-alcoholic steatohepatitis, CHB: Chronic hepatitis B infection

Table 3. Baseline Factors associated with significant fibrosis and cirrhosis in CHB patients				
Variables associated with significant fibrosis	**p**	**OR**	**95% CI Lower**	**95% CI Upper**
AST	**0.014**	1.026	1.005	1.048
GGT	**0.001**	1.022	1.008	1.035
Albumın	**0.009**	0.456	0.252	0.825
PLT	**0.001**	0.994	0.990	0.997
Variables associated with cirrhosis	**p**	OR	%95 CI Lower	%95 CI Upper
Male gender	**0.020**	4.078	1.246	13.348
GGT	**0.031**	1.013	1.001	1.025
AFP	**0.006**	1.062	1.017	1.109

(*Logistic regression analysis) AST: Aspartat aminotransferase, ALT: Alanin aminotransferase, PLT: Platelet count, GGT: Gama glutamil transferase, AFP: Alfafeto protein, INR: International normalized ratio, OR: Odds ratio, CI: Confidence interval

3.2 Cirrhosis

Statistically significant difference was found between the groups F≥5 and F<5 (cirrhosis vs non-cirrhosis) in terms of APGA, FIB-4, FI, FibroQ, S-index, APRI, PAPAS, GPR distributions (Mann-Whitney U, $p<0.05$). The mean values of these indicators were significantly higher in the cirrhosis group. The diagnostic performance of these indicators was evaluated by ROC analysis. Diagnostic performance of S-index (AUROC: 0.841), GPR (AUROC: 0.833), APGA (AUROC: 0.819) and FIB-4 Index (AUROC: 0.802) were good, APRI (AUROC: 0.767), FibroQ (AUROC: 0.700) were moderate and PAPAS (AUROC: 0.697), FI (AUROC: 0.620) were poor. Cut off points, sensitivity, specificity, PPV, NPV, positive and negative LR of these markers in diagnosing cirrhosis are shown in Table 5. ROC analysis of non-invasive markers are shown in Figure 3A, B.

Table 4. Diagnostic performance of non-invasive fibrosis markers in diagnosing significant fibrosis

İndexes	Diagnostic scan							ROC curve		p
	Cut off	Sensitivity	Specificity	PPV	NPV	LR +	LR-	Area	95% CI	
FIB-4	1.1087	50.68	77.95	72.3	58.2	2.5	0.64	0.679	0.628-0.730	**<0.001**
Lower	0.2893	99.55	0.51	53.1	50.0	1.0	0.88			
Upper	4.122	1.36	99.49	75.0	47.1	2.65	0.99			
FibroQ	1.6145	57.92	56.92	0.74	60.4	1.34	0.74	0.585	0.530-0.640	**0.002**
Lower	0.3235	99.55	1.03	53.1	50.0	1.0	0.88			
Upper	8.2984	0.45	99.49	50.0	46.7	0.88	1.0			
S-index	7.3051	52.94	77.84	73.1	59.2	2.39	0.60	0.683	0.632-0.733	**<0.001**
Lower	1.8469	99.55	2.58	53.8	83.3	1.03	0.13			
Upper	26.956	9.95	99.48	95.7	49.2	19.3	0.91			
APRI	0.4212	54.75	77.95	73.8	60.3	2.48	0.58	0.679	0.628-0.731	**<0.001**
Lower	0.1235	99.55	2.05	53.5	80.0	1.02	0.22			
Upper	1.9079	6.79	99.49	93.8	48.4	13.2	0.94			
PAPAS	0.417	59.36	60.31	62.8	56.8	1.50	0.67	0.606	0.551-0.660	**<0.001**
Lower	0.3171	99.54	0.52	53.0	50.0	1.00	0.89			
Upper	0.5337	1.83	99.48	80.0	47.3	3.54	0.99			
GPR	0.2454	45.25	80.61	72.5	56.6	2.33	0.69	0.667	0.616-0.718	**<0.001**
Lower	0.0669	99.55	3.57	53.8	87.5	1.03	0.13			
Upper	0.9081	7.24	99.49	94.1	48.7	14.2	0.93			

AUROC: Area under ROC curve, NPV: Negative predictive value, PPV: Positive predictive value, LR: Likelihood ratio, FibroQ: Fibro-quotient, FIB-4: Fibrosis index based on the four factors, GPR: GGT to Plateler Ratio, APRI: AST to Platelet ratio index, PAPAS: Platelet-Age-Phosphatase-Alfa Fetoprotein-Aspartate transaminase Index, CI: Confidence interval

Table 5. Diagnostic performance of non-invasive fibrosis markers in diagnosing cirrhosis

İndexes	Diagnostic scan							ROC Curve		p
	Cut off	Sensitivity	Specificity	PPV	NPV	LR +	LR-	Area	95% CI	
APGA	0.8886	86.21	65.12	15.6	98.4	2.47	0.21	0.819	0.751-0.888	**<0.001**
Lower	0.8058	96.55	39.02	10.6	99.3	1.58	0.088			
Upper	1.3507	3.45	99.74	50.0	93.2	13.3	0.97			
FIB-4	1.1095	86.21	66.67	16.2	98.5	2.59	0.21	0.802	0.730-0.874	**<0.001**
Lower	0.7178	96.55	32.04	9.6	99.2	0.11	9.6			
Upper	4.0348	3.45	98.97	20.0	93.2	3.34	0.98			
FI	10.34	62.07	65.37	11.8	95.8	0.58	11.8	0.620	0.504-0.736	**0.043**
Lower	8.83	96.55	5.68	7.1	95.5	1.02	0.64			
Upper	11.44	3.45	99.74	50.0	93.2	13.3	0.97			
FibroQ	1.6013	82.76	49.61	11.0	97.5	0.35	11.0	0.700	0.612-0.787	**<0.001**
Lower	0.9798	96.55	23.0	8.6	98.9	1.25	0.15			
Upper	7.7429	3.45	99.22	25.0	93.2	4.45	0.97			
S-İndex	7.9225	93.10	69.69	18.8	99.3	3.07	0.09	0.841	0.782-0.900	**<0.001**
Lower	4.9331	96.55	36.27	10.2	99.3	1.51	0.09			
Upper	74.8364	3.45	99.48	33.3	93.2	6.66	0.97			
APRI	0.4861	79.31	71.06	17.0	97.9	2.74	0.29	0.767	0.687-0.846	**<0.001**
Lower	0.2533	96.55	30.75	9.5	99.2	1.39	0.11			
Upper	3.3516	3.45	98.97	20.0	93.2	3.34	0.98			
PAPAS	0.4167	85.71	51.95	11.5	98.0	1.78	0.28	0.697	0.615-0.780	**<0.001**
Lower	0.3743	96.43	21.82	8.2	98.8	1.23	0.16			
Upper	0.5443	3.57	99.74	50.0	93.4	13.7	0.97			
GPR	0.2558	86.21	72.94	19.2	98.6	3.19	0.19	0.833	0.769-0.898	**<0.001**
Lower	0.1329	96.55	33.51	9.8	99.2	1.45	0.10			
Upper	1.8606	3.45	99.74	50.0	93.3	13.4	0.97			

AUROC: Area under ROC curve, NPV: Negative predictive value, PPV: Positive predictive value, LR: Likelihood ratio, APGA index: AST/Platelet/GGT/Alpha-fetoprotein Index, FI: Fibrosis Index, FibroQ: Fibro-quotient, FIB-4: Fibrosis index based on the four factors, GPR: GGT to Plateler Ratio, APRI: AST to Platelet ratio index, PAPAS: Platelet-age-phosphatase-alfa Fetoprotein-Aspartate Transaminase Index, CI: Confidence interval

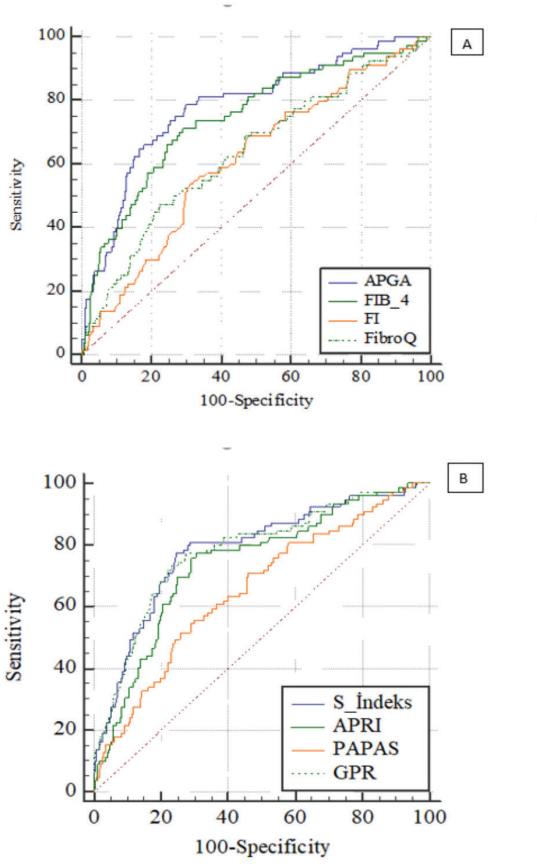

Figure 3. Receiver operating characteristic analysis of non invasive markers in the prediction of cirrhosis (A-B)

APGA Index: AST/Platelet/GGT/Alpha-fetoprotein Index, FIB-4: Fibrosis Index based on four factors, FI: Fibrosis Index, FibroQ: Fibro-quotient, APRI: Aspartate transaminase to-Platelet Ratio Index, PAPAS: Platelet-Age-phosphatase-alfa fetöprotein - aspartate transaminase, GPR: GGT Gama-glutamil transferaz to platelet ratio

Discussion

In the management of CHB, the grade of liver fibrosis is an important determinant of prognosis and timing of the treatment decision. Liver biopsy is the gold standard for detecting fibrosis, however the procedure is invasive, costly and not always repeatable. Furthermore, biopsy may not accurately reflect the stage of fibrosis due to heterogeneous distribution and a small sampling size (19). In addition, biopsy material should be evaluated by experienced pathologists (11). For this reason, studies are carried out to determine fibrosis grade by the non-invasive methods. In this study, the diagnostic performance of simple non-invasive fibrosis markers (APGA, FI, FIB-4, FibroQ, S-index, APRI, AAR and GPR) were evaluated.

We found that S-index, APRI, FIB-4 Index, GPR, PAPAS and FibroQ indicators can detect accurately significant fibrosis. In diagnosis of significant fibrosis, the AUROC value of the S-index was higher than the other non-invasive indicators, but the diagnostic performance of the S-index and others were poor (AUROC <0.700). PPV, AUROC and positive likelihood ratio values of S-index, APRI, FIB-4, GPR Index were found close to each other. If these tests are used at optimal cut-off points, 26-28% of patients can be diagnosed to have significant fibrosis as false positive.

In addition, we found that the AUROC value of the S-index in the diagnosis of cirrhosis was high (AUROC: 0.841), and the diagnostic performance was better than the other non-invasive indicators. Along with S-index, we also found that GPR (AUROC: 0.833), APGA (AUROC: 0.819) and FIB-4 (AUROC: 0.802) indexes had good diagnostic performance in detecting cirrhosis. If these tests are used for a value at or below the optimal cut-off point to exclusion of cirrhosis, %98-99 of patients will be determined correctly.

The APRI and FIB-4 Indexes are non-invasive fibrosis indicators used firstly in patients with HCV or HCV/HIV co-infection in the Western population. In 2015, a meta-analysis evaluated the diagnostic performance of APRI, FIB-4 Index in CHB, reported that AUROC values were found to be 0.740, 0.784 for significant fibrosis and 0.726, 0.844 for cirrhosis respectively (20). In 2015, the WHO recommended the use of the APRI score (APRI score >2 in adults) to assessing the presence of cirrhosis where source limited settings in CHB patients (21). However, in our study, diagnostic performance of APRI was not as good as S-index, GPR, APGA and FIB-4 for determining cirrhosis.

Zhou et al. (13) reported that S-index has good diagnostic performance in detecting significant fibrosis (AUROC: 0.812) and cirrhosis (AUROC: 0.890) (14). Also, Tag-Adeen et al. (22) confirmed this result (AUROC: 0.810) in diagnosing significant fibrosis and reported that the S-index was excellent in the diagnosis of cirrhosis (AUROC: 0.960), superior to the APRI, FIB-4 Index. In our study, the diagnostic performance of the S-index was not as good as the results reported by Zhou et al. (13) and Tag-Adeen et al. (22) These inconsistencies may be related to different demographic, viral characteristics of the study groups and ethnic differences. Also, in our study, liver fibrosis was evaluated with Ishak score but Zhou et al. (13) and Tag-Adeen et al. (22) used Scheuer's and Metavir scores, respectively. ISHAK score documents the minimal changes in fibrosis stage better than other classifications and evaluates fibrosis as 7 stages (23,24).

Lemoine et al. (16) reported that the diagnostic performance of GPR was superior to the APRI and FIB-4 Index in the diagnosis of significant fibrosis (AUROC: 0.720 and 0.730 in different cohorts) and cirrhosis (AUROC: 0.830 and 0.870) in CHB patients (17). However, in a recent meta- analysis it has been shown that, GPR has moderate diagnostic accuracy for predicting HBV-related significant fibrosis, severe fibrosis, and cirrhosis (AUROC values 0.733, 0.777, and 0.796, respectively) (25). Our study demonstrated that diagnostic performance of GPR was poor in significant fibrosis and good in cirrhosis. Different from our study, the use of elastography as a reference in the study of Lemoine et al. (16) may explain the inconsistency of results between studies.

Our study demonstrated that APGA, FI and AAR Idexes could't determinated the significant fibrosis, also AAR could't diagnosed the cirrhosis. In addition, FibroQ, PAPAS and FI scores were found to be weaker than other indexes to diagnosis of cirrhosis.

Study Limitations

Retrospective and single centre design are limitation of our study. İn adition, the distribution of most patients between fibrosis stage 2,3 might have negatively affect the diagnostic performance results of non-invasive tests.

Conclusion

We found that the diagnostic performance of S-index for diagnosing cirrhosis and significant fibrosis was superior to GPR APRI, FIB-4 Index, FibroQ, PAPAS, AAO, APGA and FI indices in patients with CHB. However, diagnostic performance of S-index, GPR, APRI, FIB-4, FibroQ, PAPAS indices were poor in predicting significant fibrosis (AUROC <0.700). Therefore, we believe that these indirect non-invasive fibrosis indicators have limited value for diagnosing significant fibrosis. The diagnostic performance of S-index, APGA, GPR, and FIB-4 Index were good for excluding cirrhosis. We think that these indexes can be used to excluding CHB related cirrhosis in source limited regions.

Ethics

Ethics Committee Approval: The ethics committee approval for the study was obtained from the Local Ethics Committee of Ümraniye Training and Research Hospital (approval number: B10.1TKH.4.34.H.GP0.01/34, date: 20/02/2019).

Informed Consent: Couldn't obtained due to retrospective design.

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Authorship Contributions

Surgical and Medical Practices: K.K., R.K., Z.Ç., S.S., H.D., Gü.A., R.A., Concept: S.S., Design: S.S., Data Collection or Processing: S.S., R.A., Ş.Ç., K.K., Gü.A., Analysis or Interpretation: G.A., K.Ö., S.S., Literature Search: R.A., H.L.D., O.Ö., Writing: S.S.

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Research Article

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Age Specific Hepatitis B Surface Antigen (HBsAg) and Anti-HBs Seroprevalence among Patients Admitted to a State Hospital

Devlet Hastanesine Başvuran Hastalarda Yaşa Özgü Hepatit B Yüzey Antijeni ve Anti-HBs Seroprevalansı

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ABSTRACT

Objectives: Chronic hepatitis B (CHB) infection affects 257 million people globally, Turkey is middle endemic region. According to public health data, about 2 million people in Turkey are considered to be positive for hepatitis B surface antigen (HBsAg). With effective vaccination programs, active and passive immunizations of newborns born from infected mothers and increased sanitation, the prevalence of HBsAg has declined over the past years. In Turkey, Hepatitis B virus vaccine has been included in the national vaccination schedule in 1998. In the study, our aim is to compare the seroprevalence of HBsAg and Anti-HBS by birth-date in Sungurlu State Hospital, Çorum.

Materials and Methods: The results of 1,486 patients who applied to our hospital were scanned retrospectively on the electronic system. The rate of HBsAg and anti-HBs seropositivity were compared by birth-date.

Results: Of 1486 cases, 737 were women and 749 were men. The median age was 38 (0-95 years). HBsAg positivity was 1.9% and anti-HBs positivity was 54.2%. All of 27 HBsAg positive patients were born before 1998. HBsAg positivity was found almost 3 times higher in the male population. Anti-HBs positivity was 41% in patients born before 1990, and 71% in those born in 1998 and after.

Conclusion: Immunization is very important and effective way in chronic hepatitis B infection control and prevention. The results of our study showed a significant decrease in CHB infection with the national vaccination program. It is very essential to administer HBV vaccination to all people, especially those at risk.

Keywords: HBsAg, anti-HBs, seroprevalence

ÖZ

Amaç: Kronik hepatit B (KHB) enfeksiyonu dünya genelinde 257 milyon kişiyi etkilemektedir, Türkiye orta endemiktir. Halk sağlığı verilerine göre, Türkiye'de yaklaşık 2 milyon kişinin hepatit B yüzey antijeni (HBsAg) pozitif olduğu düşünülmektedir. Etkili aşılama programları, HBsAg pozitif anneden doğan yeni-doğanların aktif ve pasif immünizasyonu ve artan hijyen koşullarıyla beraber son yıllarda HBsAg prevalansı azalmaktadır. Türkiye'de hepatit B aşısı 1998'de ulusal aşı programına alınmıştır. Bu çalışmada, Çorum Sungurlu Devlet Hastanesi'nde takip edilen hastalarda HBsAg ve anti-HBs seroprevalansının doğum yılına göre karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Hastanemizde takip edilen 1.486 hastanın sonuçları retrospektif olarak elektronik sistem üzerinden tarandı. HBsAg ve anti-HBs seropozitifliği oranı doğum yılı ile karşılaştırıldı.

Bulgular: Bin dört yüz seksen altı hastanın 737'si kadın, 749'u ise erkekti. Ortanca yaş 38 (0-95 yaş) idi. HBsAg pozitifliği %1,9 anti-HBs pozitifliği ise %54,2 olarak bulundu. HBsAg pozitif bulunan 27 hastanın tamamı 1998 öncesi doğmuştu. HBsAg pozitifliği erkeklerde 3 kat daha fazla bulundu. Anti-HBs pozitifliği, 1990 öncesinde doğanlarda %41 iken 1998 ve sonrasında doğanlarda %71 idi.

Sonuç: Bağışıklama, hepatit B enfeksiyonunun kontrolünde ve önlenmesinde çok önemli ve etkili bir yoldur. Çalışmamızın sonuçları, ulusal aşılama programı ile KHB enfeksiyonunda önemli bir azalma olduğunu göstermiştir. Hepatit B aşılamasının tüm insanlara özellikle de risk grubundakilere uygulanması çok önemlidir.

Anahtar Kelimeler: HBsAg, anti-HBs, seroprevalans

Kaya SY, Abdurrahman Kaya A. Age Specific Hepatitis B Surface Antigen (HBsAg) and Anti-HBs Seroprevalence among Patients Admitted to a State Hospital. Viral Hepat J. 2020;26:85-87.

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Introduction

Chronic hepatitis B (CHB) infection is a common liver disease that affects 257 million people globally (1). The prevalence of hepatitis B surface antigen (HBsAg) ranges from 0.7 to 6.8% worldwide. Turkey is middle endemic region for CHB infection, according to World Health Organization (WHO) data.

With effective vaccination programs, active and passive immunizations of newborns born from infected mothers and increased sanitation, the prevalence of HBsAg has declined over the past years. However, it still remains one of the most important causes of liver cirrhosis and hepatocellular carcinoma (HCC) all over the world, especially in underdeveloped countries. In a systematic review between 1999-2009, HBsAg positivity was found to be 4.6% in our country (2). Currently, according to public health data, about 2 million people in Turkey (2% of the population) are considered to be positive for HBsAg (3).

In Turkey, Hepatitis B virus (HBV) vaccine has been included in the national vaccination schedule in 1998. And catch-up immunization has been performed in primary schools. Thereby the vast majority of the generation born after 1990 has been vaccinated.

In the study, our aim is to compare the seroprevalence of HBsAg and anti-HBS by birth-date in Sungurlu State Hospital, Çorum.

Materials and Methods

The results of patients who applied to our hospital between January 2018 and October 2019 for any reason and who tested for HBsAg and anti-HBs were scanned retrospectively on the electronic system. As our study was retrospective, ethical approval and patients consent were not obtained. A total of 1486 patients were evaluated. The patients were divided into three groups according to their birth dates; 1. born before 1990 (918 cases), 2. born between 1991-1997 (216 cases), 3. born in 1998 and after (352 cases). The rate of HBsAg and Anti-HBs seropositivity were compared by birth-date.

Statistical Analysis

SPSS 21 program was used to analyze the data. Chi-square and Mann-Whitney U were used to compare categorical and continuous variables respectively. Post-hoc analysis with bonferroni correction was used for statistically significant results in the presence of more than two groups. P-value ≤0.05 was considered significant.

Results

Of 1486 cases, 737 were women and 749 were men. The median age was 38 (0-95 years). HBsAg positivity was 1.9% (29) and anti-HBs positivity was 54.2% (805). While 27 of the HBsAg positive patients were born before 1990, 2 patients were born in between 1990-1998. HBsAg positivity was found almost 3 times higher in the male population (Table 1). Of 29 patients with HBsAg positivity, 21 were male and 8 were female, the difference between the genders was statistically significant (p=0.02). Eight patients were inactive HBsAg carriers and 7 were under antiviral treatment with the diagnosis of CHB. Fourteen patients (48%) were without follow-up. Anti-HBs positivity was 41% in patients born before 1990, and 71% in those born in 1998 and after (p>0.001). Anti-HBs positivity was slightly higher in men (Table 1), but not statistically significant (p=0.10). Anti-HCV positivity was 0.4% (6/1447) and anti-HIV positivity was 0.1% (2/1332).

Discussion

With effective vaccination programs, although the incidence of HBsAg has been decreasing all over the world, CHB is still the most important cause of liver cirrhosis and HCC-related deaths. It is estimated 887.000 deaths per year (1). In Turkey, approximately 2% of the population are considered to be HBsAg positive, and 40-50% of liver transplantations between 2012 and 2016 were caused by complication of HBV infection (3,4). On the other hand, as the disease remains silent without having complication, most patients are unaware of their illness. While, only 10.5% of HBsAg positive individuals are thought to be aware of their disease worldwide, this rate was found 12% in a study in Turkey (1,5). In our study, 14 of 29 HBsAg positive patients (48%) did not have CHB follow-up.

Vaccination is the most effective way to control and prevent the disease. HBV vaccination provides 98-100% protection from HBV infection. In 1992, WHO called for vaccination of infants against HBV worldwide, thereafter, vaccination programs for HBV have been conducted in most countries. In Turkey, HBV vaccine has been included in the national vaccination program since 1998. According to Public Health Agency data, the rate of vaccination for hepatitis B increased from 64% in 1999 to 98% in 2018 in Turkey. While the incidence of acute hepatitis B was 4-6 per 100.000 in 1990, it was reported as 1.9 per 100.000 in 2017 in our country. Under the age of 15, this rate has decreased to less than 1 per 100.000 (3).

HBV vaccine is the first vaccine that prevent from cancer. In a study published in 1997 in Taiwan, it was observed that there

Table 1. Distribution of hepatitis B surface antigen (HBsAg) and anti-HBs positivity by gender and birth-year

		Number of cases	HBsAg (n, %)	Anti-HBs (n, %)
Gender	Female	737	8 (1)	385 (52)
	Male	749	21 (2.8)	420 (56)
Birth-year	≤1989	918	27 (2)	383 (41)
	1990-1997	216	2 (0.9)	169 (78)
	≥1998	352	0 (0)	253 (71)
Total	-	1486	29 (1.9)	805 (54.2)

HBsAg: Hepatitis B surface antigen

was a significant decrease in childhood HCC cases after the HBV vaccination program (6). In another study from Alaska, it was emphasized that there was a significant decrease in acute hepatitis B and HCC cases in childhood after the national vaccination program (7).

The results of our study showed a significant decrease in CHB infection with the national vaccination program. HBsAg positivity was found 1.9% in our study group, and all patients were born before 1998. The results were consistent with the rate of our country (2%). HBsAg positivity was found 2.5% in a study including 61.943 volunteers from 73 provinces (8). In another study from Turkey, HBsAg was found positive in 4.75% of 58.752 cases and 97% of the cases were born before 1998 (9). In a review HBsAg positivity in pregnant women between 1975-2016, it was seen that HBsAg positivity varied between 1.2-19.2%. This rate was decreased to 1.2-5.2% after 2010 (10).

While the anti-HBs positivity was found to be about 70% in the first 3 decades, it decreased below 40% in the subsequent decades. As of the 7th decade, it seems that anti-HBs positivity started to increase again (Table 2). This increase is thought to be associated with increased disease contact. In a study published in 2016, similar to our study, anti-HBs positivity was 85.56% in the 0-12 age group and 56.4% in the 13-20 age group, it decreased to 20% within the 20 years. In those over 50 years old, anti-HBs positivity increased again and rises above 40% (11).

Study Limitations

This study had some limitations including retrospective design, low number of cases and unknown of patients anti-HBc IgG status. Prospective studies are needed to better demonstrate these findings.

Conclusion

HBV infection is a communicable disease that can have fatal complications. Immunization is very important and effective way in its control and prevention. With national vaccination program, it is clear that the incidence of the disease has decreased considerably. Therefore, it is very essential to administer HBV vaccination to all people, especially those at risk.

Table 2. Distribution of anti-hepatitis B surface positivity by ages

Age	Number of cases	Anti-HBs positivity (n, %)
0-10	140	100 (71.4)
11-20	181	125 (69.1)
21-30	274	203 (74.1)
31-40	204	76 (37.3)
41-50	179	59 (33)
51-60	157	60 (38.2)
61-70	167	79 (47.3)
71-80	128	67 (52.3)
81-90	48	30 (62.5)
91-100	8	6 (75)
Total	1486	805 (54.2)

HBs: Hepatitis b surface

Ethics

Ethics Committee Approval: As our study was retrospective, ethical approval was not obtained.

Informed Consent: Patient consent was not obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.Y.K, A.K., Concept: S.Y.K, A.K., Design: S.Y.K, A.K., Data Collection or Processing: S.Y.K, A.K., Analysis or Interpretation: S.Y.K, A.K., Literature Search: S.Y.K, A.K., Writing: S.Y.K, A.K.

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Research Article

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An Evaluation of the Efficacy of High-dose Hepatitis B Vaccine in Patients Using Biological Agents

Biyolojik Ajan Kullanan Hastalarda Yüksek Doz Hepatit B Aşısının Etkinliğinin Değerlendirilmesi

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ABSTRACT

Objectives: Hepatitis B virus (HBV) vaccination is efficient in the normal population, whereas lower humoral response rates in immunosuppressed patients. Biological agents used in the treatment of several diseases in recent years are a significant cause of immunosuppression in patients. In this study we aimed to evaluate the efficacy of double-dose HBV vaccination at months 0, 1, 2, and 6 in patients using biological agents.

Materials and Methods: The patients who were using biological agents and seronegative for HBV received double-dose HBV vaccine (40 µg) on months 0, 1, 2 and 6, and response rates were assessed. Patients with anti-HBs titers >10 mIU/mL one month after completion of the vaccine plan were regarded as vaccine-responsive.

Results: Eighty-four patients were evaluated. Forty patients (47.4%) were men and 44 (52.4%) were women. The mean age of the patients was 43.1±12.5 years. The most common underlying inflammatory rheumatic disease was ankylosing spondylitis at 51.2% (n=43). The most commonly used biological agent was adalimumab at 36.9% (n=31). Vaccine response was achieved in 85.7% (n=72) of the patients, while no response was achieved in 12 patients (14.3%). Sex, comorbidities, type of underlying inflammatory disease and biological agents had no effect on vaccine response.

Conclusion: Administration of 40 µg HBV vaccine at months 0, 1, 2, and 6 to HBV seronegative patients using biological agents was found to be effective. This efficacy was found to be independent of the type of biological agent, the time of onset of the biological agent and the length of use of the biological agent.

Keywords: Hepatitis B vaccines, biological factors, vaccination, hepatitis B virus, rheumatic diseases

ÖZ

Amaç: Hepatit B virus (HBV) aşısı normal popülasyonda etkin iken, immünosüpresif hastalarda humoral yanıt oranları daha düşüktür. Son yıllarda birçok hastalıkta kullanılan biyolojik ajanlar önemli bir immünosüpresyon nedenidir. Bu çalışmada, biyolojik ajan kullanan hastalarda 0, 1, 2 ve 6. aylarda çift doz HBV aşılamasının etkinliğinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Biyolojik ajan kullanan ve HBV için seronegatif olan hastalara 0, 1, 2 ve 6. aylarda çift doz (40 µg) HBV aşısı uygulanarak yanıt oranları değerlendirildi. Aşı şeması tamamlandıktan bir ay sonra bakılan anti-HBs titresi >10 mIU/mL olan hastalar aşı yanıtlı olarak kabul edildi.

Bulgular: Seksen dört hasta değerlendirildi. 40 hasta (%47,4) erkek ve 44 hasta (%52,4) kadındı. Hastaların yaş ortalaması 43,1±12,5 idi. Altta yatan enflamatuvar romatizmal hastalıklarından en sık görüleni %51,2 (n=43) oranında ankilozan spondilit idi. Biyolojik ajanlardan en sık %36,9 (n=31) oranında adalimumab kullanılmıştı. Çalışmada hastaların %85,7'sinde (n=72) aşı yanıtı sağlanırken 12 hastada (%14,3) aşı yanıtı alınamadı. Cinsiyetin, komorbiditelerin ve altta yatan enflamatuvar romatizmal hastalığın ve biyolojik ajanın türünün aşı yanıtını etkilemediği saptandı.

Sonuç: Biyolojik ajan kullanan HBV seronegatif hastalarda; 0, 1, 2 ve 6. aylarda 40 µg HBV aşı uygulamasının etkin olduğu saptanmıştır. Bu etkinliğin biyolojik ajanın türünden, biyolojik ajana başlama zamanından ve biyolojik ajanı kullanma süresinden bağımsız olduğu görülmüştür.

Anahtar Kelimeler: Hepatit B aşıları, biyolojik faktörler, aşılama, hepatit b virüsü, romatolojik hastalıklar

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Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. Chronic HBV infection is a frequent cause of cirrhosis and hepatocellular cancer (1).

Since HBV is a disease that can be prevented by vaccination, contemporary guidelines recommend that high-risk groups should be screened and immunized. According to these guidelines, all patients considered for immunosuppressive therapy should be screened for HBV, and HBV-seronegative patients must be vaccinated. It is also recommended that, if possible, vaccination should be initiated before immunosuppressive therapy (2,3,4,5,6). The risk of HBV reactivation varies depending on the class of immunosuppressive therapy. Cancers and inflammatory and autoimmune diseases play a determining role in the incidence of HBV reactivation (5). Many drugs used in the treatment of these diseases such as antimetabolites, tumor necrosis factor (TNF) inhibitors, steroids, systemic chemotherapy and biological agents lead to immunosuppression (7).

Biological agents are drugs that inhibit specific molecules or cellular targets in the pathogenesis of diseases. They make a positive contribution to prognosis by targeting TNF, interleukin-1 and 6, and cytotoxic T lymphocyte antigen-4 and B cells (8). Biological agents have become increasingly used in the treatment of inflammatory rheumatic diseases, and are a significant cause of immunosuppression in these patients (7).

Humoral response rates to HBV vaccination are >90% in the normal population, but lower in immunosuppressed patients (9). In some studies, response rates were found between 34-49% in immunosuppressive patients (10,11,12). Higher-dose or reinforced vaccines may be required to achieve anti-hepatitis B surface (HBs) response in immunosuppressed patients (13). Antigen specific B and T lymphocytes play important roles in the antibody response to HBV vaccine (14). TNF inhibitors suppresses the T-cell and B-cell mediated immune response (15,16). The Centers for Disease Control and Prevention currently recommends that since vaccine response is low in patients receiving immunosuppressive therapy, vaccination should be applied in a double dose (two concomitant adult HBV vaccine doses) at months 0, 1, 2 and 6 (17).

There are very few studies in the literature evaluating the HBV vaccine response in patients using biological agents. In these studies, it was found that the vaccine response decreased in patients using biological agent therapy (15,18). However, no study evaluating the effectiveness of the high-dose vaccine administered four times was found. Regardless of the type of biological agent used in our study, all patients were administered double dose HBV vaccine for 4 times. The purpose of this study was to evaluate the efficacy of double-dose HBV vaccination at months 0, 1, 2, 6 and to evaluate the factors affecting the vaccine response in patients using or scheduled to be started on biological agents due to underlying diseases.

Materials and Methods

The patients who were using biological agents and followed up in infectious diseases outpatient clinic between January 2017 and July 2018 were evaluated retrospectively in this study.

Hepatitis B surface antigen (HBsAg), hepatitis B core antibody immunoglobulin G, and anti-HBs were investigated using the Enzyme-Linked Immunosorbent Assay method (Roche, Hitachi- Cobas 6000). Patients seronegative for HBV received double-dose HBV vaccine (40 µg) on months 0, 1, 2 and 6, and response rates were assessed. Vaccination was performed both on patients already started on biological agents and on those scheduled to begin using such agents within the following two weeks. Patients' demographic data, underlying diseases, and comorbidities were recorded. Patients' anti-HBs titers were investigated one month after completion of the vaccine schedule. Subjects with anti-HBs titers >10 mIU/mL were regarded as vaccine-responsive.

The study protocol was approved by Karadeniz Technical University Scientific Research Ethics Committee (approval number: 2019-253). Informed consent wasn't obtained.

Statistical Analysis

SPSS 23.0 software was used for statistical data analysis. Student's t test was employed to compare numerical variables between two independent groups, and the chi-square test in the comparison of qualitative data. P-values <0.05 were regarded as statistically significant.

Results

Eighty-four patients who had received vaccination were evaluated. Forty patients (47.4%) were men and 44 (52.4%) were women. The mean age of the patients was 43.1±12.5 years, with a median value of 42 years. The most common underlying inflammatory rheumatic diseases were ankylosing spondylitis at 51.2% (n=43), followed by psoriasis at 25% (n=21), and rheumatoid arthritis at 22.6% (n=19). Reactive arthritis was diagnosed in only one patient. Patients' underlying diseases, comorbidities and biological agents are summarized in Table 1.

The most commonly used biological agent was adalimumab at 36.9% (n=31). No relation was observed between biological agents used and patients vaccine responses (p=0.152).

HBV vaccination was performed a mean two weeks before biological agent use in 43 (51.2%) patients, and during biological agent use in 41 (48.8%). These patients had been using biological agents for a median 36 months. Comparison of these two groups revealed response rates of 81.4% in patients started on vaccine before biological agent use and of 90.2% in those starting vaccine during biological agent use. The difference between the two groups' vaccine responses was not statistically significant (p=0.397).

Thirty-nine (46.4%) patients were using immunomodulatory therapies such as methotrexate and prednisolone before starting on biological agents (Table 1). Use of these therapies prior to biological agents had no effect on vaccine response (p=0.392).

Vaccine response in the form of anti-HBs>10 mIU/mL was achieved in 85.7% (n=72) of the 84 patients receiving HBV vaccine, while no response was achieved in 12 patients (14.3%). Anti-HBs levels in the patients with vaccine response ranged between 19 and 1000 mIU/mL, with a mean value of 740.9±379.9 mIU/mL. The relation between anti-HBs level and biological agent used in patients with vaccine response is shown in Figure 1. Vaccine response was higher in young patients than elders (p=0.049). Sex, comorbidities, and type of underlying inflammatory disease had no effect on vaccine response (Table 2).

Table 1. Clinical and demographic characteristics of patients	
Characteristics of patients (n=84)	
Mean age	43.1±12.5
Median age	42
Gender	n (%)
Male	40 (47.6)
Female	44 (52.4)
The underlying rheumatic disease	
Rheumatoid arthritis	19 (22.6)
Anklylosing spondylitis	43 (51.2)
Psoriasis	21 (25)
Reactive arthritis	1 (1.2)
Comorbid diseases	
Diabetes mellitus	5 (6)
Hypertension	6 (7.1)
Coronary artery disease	3 (3.6)
Chronic lung disease	3 (3.6)
Hypothyroidism	3 (3.6)
Biological agents	
Infliximab	10 (11.9)
Adalimumab	31 (36.9)
Etanercept	21 (25)
Ustekinumab	3 (3.6)
Tofacitinib	7 (8.3)
Golimimab	7 (8.3)
Tocilizumab	3 (3.6)
Sertolizumab	1 (1.2)
Abatacept	1 (1.2)
Drug used before biological agent	
Prednisolone	3 (3.6)
Methotrexate	12 (14.3)
Prendisolone and methotrexate	12 (14.3)
Infliximab	2 (2.5)
Etanercept	4 (4.8)
Abatacept	1 (1.2)
Adalimumab	1 (1.2)
Result	
Vaccine responsive	72 (85.7)
Vaccine unresponsive	12 (14.3)
The mean level of anti-hepatitis B surface in vaccine responders (IU/l)	**740.9 ± 379.9**

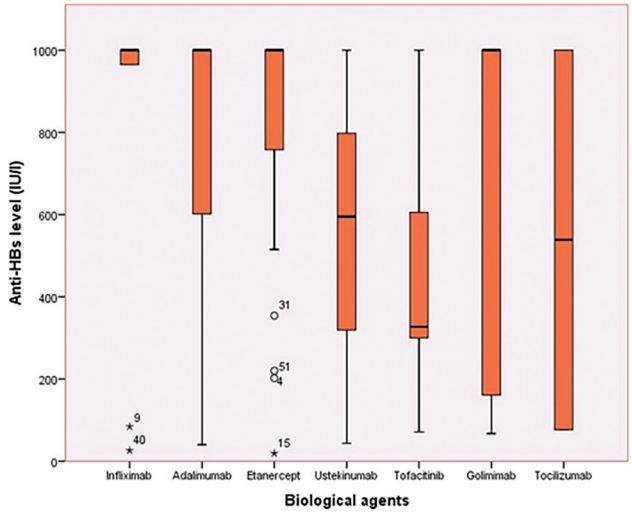

Figure 1. Relation between biological agents and anti-hepatit B surface titers in patients with vaccine responders
Anti-HBs: Anti-hepatit B surface

Discussion

HBV infection is a serious global health problem. Approximately 6% of the world population is chronically infected with HBV (19). HBV is widely transmitted by body fluids such as blood, sperm, and vaginal secretions. The most effective method for protection against this infection is vaccination (17). With its "Global Health Sector Strategy" announced in 2016, the World Health Organization aims for the elimination of viral hepatitis by 2030, and vaccination is the best way to achieve that (20). Thanks to universal HBV vaccination in newborns, the epidemiology of chronic HBV infection has altered dramatically in several parts of the world (19). Due to HBV vaccination at 0, 1, and 6 months, the response rate in healthy adults under 40 now exceeds 90%, although response rates are known to decrease with age. Response rates are lower in immunosuppressed patients (21). Various strategies have been developed in order to increase vaccine response rates in subjects with some chronic diseases or receiving immunosuppressive therapy, including increasing the vaccine dosage, intradermal administration, alternative adjuvants, alternative routes of administration, concomitant administration with other vaccines, and novel therapies (22). One of the methods employed to increase vaccine response in immunosuppressive patients is double-dose (40 µg) HBV vaccination at months 0, 1, 2 and 6 (17).

Biological agents that have become increasingly used in the treatment of inflammatory rheumatic diseases are an important cause of immunosuppression in these patients (7). There are no specific recommendations regarding HBV vaccination in patients using biological agents. As set out in the current guidelines patients receive immunosuppressive therapy must be screened for HBV, and HBV seronegative subjects must be immunized. The guidelines also state that commencing immunization before immunosuppressive therapy increases antibody response (2-4). Two weeks are required for immune response to develop in inactive vaccines. It is therefore recommended that immunization commence two weeks prior to the start of immunosuppressive therapy. However, when immunosuppressive therapy is completed, the timing of vaccination may vary depending on the biological agent employed (23). In contrast to other guidelines, the American College of Rheumatology guideline strongly recommends HBV vaccination in patients with rheumatic arthritis already using biological agents. The reason for this recommendation in the

Table 2. Comparison of vaccine responder and non-responder patients

Characteristics of patients	Anti-HBs positive (n=72, 85.7%)	Anti-HBs negative (n=12, 14.3%)	p
Mean age	42.01±11.75	49.67±15.38	**0.049**
Median age	41.5	51	
Gender	n (%)	n (%)	
Female	40 (55.6%)	4 (33.3%)	0.265
Male	32 (44.4%)	8 (66.7%)	
The underlying rheumatic disease			
Rheumatoid arthritis	13 (18.1%)	6 (50%)	0.108
Anklylosing spondylitis	39 (54.2%)	4 (33.3%)	
Psoriasis	19 (26.4%)	2 (16.7%)	
Reactive arthritis	1 (1.4%)	0	
Comorbidities			
Diabetes mellitus	4 (5.6%)	1 (8.3%)	0.743
Hypertension	5 (6.9%)	1 (8.3%)	
Coronary artery disease	2 (2.8%)	1 (8.3%)	
Chronic lung disease	3 (4.2%)	0	
Hypothyroidism	3 (4.2%)	0	
Biological agents			
Infliximab	10 (13.9%)	0	0.152
Adalimumab	27 (37.5%)	4 (33.3%)	
Etanercept	19 (26.4%)	2 (16.7%)	
Ustekinumab	3 (4.2%)	0	
Tofacitinib	5 (6.9%)	2 (16.7%)	
Golimimab	5 (6.9%)	2 (16.7%)	
Tocilizumab	2 (2.8%)	1 (8.3%)	
Sertolizumab	1 (1.4%)	0	
Abatacept	0	1 (8.3%)	
Start vaccination before using biological agents	35 (48.6%)	8 (66.7%)	0.397
Start vaccination while using biological agents	37 (51.4%)	4 (33.3%)	
Use of immunomodulatory drug before biological agent	29 (40.3%)	7 (58.3%)	0.392

HBs: Hepatitis B surface

guideline is the documented benefit of inactivate pneumococcal vaccine in patients with rheumatic arthritis already using biological agents and the absence of any major concern over damage (24). In our study, HBV vaccination was also administered to patients already using biological agents for a long time in addition to those schedules to receive biological agent therapy. Vaccine response analysis at the end of the study revealed no difference in responses between subjects started on vaccination before biological agent use and those started on vaccination while already using biological agents (81.4% and 90.2%, respectively). Although rituximab was not being used in our study, the type of agent employed and the duration of use had no effect on vaccine response. This shows that high-dose HBV vaccination (40 µg) at months 0, 1, 2 and 6 may be beneficial in patients using biological agents, independently of the type of agent or the length of use.

Haykir Solay and Eser et al. (10) evaluated the results of HBV vaccination in patients with inflammatory rheumatic disease using immunomodulatory therapy. Three doses of 20 µg and 40 µg HBV vaccine were applied on months 0, 1 and 6. Response rates were 49.3% in patients receiving the standard schedule, and 61.1% in the high-dose group. The difference between the two groups was not statistically significant. In addition, response rates in patients using infliximab were lower than in patients using ustekinumab and etanercept. In our study, a four-dose vaccination schedule was employed and a higher vaccine response rate was achieved, but no difference was determined in vaccine responses in terms of biological agents. However, in one study infliximab use was found not to affect the HBV vaccine response rate in children with inflammatory bowel disease (25).

A similar study comparing the efficacy of double-dose HBV vaccine administration at 0, 1, and 2 months in patients with inflammatory bowel disease reported anti-HBs >10 IU/l in 59% of patients. A response rate of 45% was determined in patients using TNF inhibitors (11). The vaccine response rate in the present

study being higher than in that study may be due to our vaccination schedule involving a further double-dose at six months.

Studies of the efficacy of high-dose HBV vaccination in immunosuppressive individuals have largely focused on HIV-positive individuals. Four prospective studies on that subject applied 40 µg HBV vaccine at 0, 1, and 6 months, with response rates ranging between 46.9% and 63.8% (12,26,27,28). Protective antibody response rates of 89.4% and 90.8% were determined n two observational studies in which 40 µg HBV vaccine was administered to HIV-positive patients at 0, 1, 2, and 6 months, (29,30). High-dose HBV vaccination has also been studied and shown to be effective in chronic kidney diseases, cancer patients receiving chemotherapy, drug abusers, nondrug-responsive subjects and cirrhosis patients (31,32,33,34,35). Although the underlying diseases are not the same, these data from the immunosuppressive patient group support our own findings.

Study Limitations

This study has several limitations. The retrospective nature of the study and the small number of patients are main limitations of the study. This study needs to be investigated in a larger number of patients to provide detailed clarification on the relationship of vaccine response with underlying rheumatic disease, age, biological agent used etc. In this study, hepatitis B vaccination was performed in double dose (40 µg) at 0, 1, 2 and 6 months. If a group of patients were given 20 µg of vaccine in the same scheme and these two groups were compared, a better contribution would be made to the literature.

Conclusion

High antibody levels were achieved with the administration of 40 µg HBV vaccine at months 0, 1, 2, and 6 to HBV seronegative patients using biological agents, independently of the type of biological agent and length of use. Our scans of databases such as Pubmed, Google Scholar, Web of Science, and Research gate revealed no similar studies of the efficacy of 20 µg or 40 µg HBV vaccine at months 0, 1, 2, and 6 in patients using biological agents. Vaccination in this patient group should not be overlooked in daily practice. Further studies with larger patient numbers comparing different vaccine doses and schedules are now needed to identify the appropriate schedule and effective dosage.

Ethics

Ethics committee approval: The study protocol was approved by Karadeniz Technical University Scientific Research Ethics Committee (approval number: 2019-253).

Informed Consent: Informed consent wasn't obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices - M.A., Concept: M.A., F.A., Design: F.A., Data Collection or Processing: Z.Y., M.A., Analysis or Interpretation: İ.K., Literature Search: M.A., F.A., Writing: M.A., F.A.

Conflict of Interest: The authors declare no conflict of interest.

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Research Article

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The Evaluation of a Hundred Eleven Adult Patients with Acute Hepatitis

Akut Hepatitli Yüz On Bir Yetişkin Hastanın Değerlendirilmesi

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ABSTRACT

Objectives: We aimed to evaluate the etiological, epidemiological and laboratory characteristics of adult patients admitted to our hospital with acute hepatitis.

Materials and Methods: The patients with alanine aminotransferase (ALT) levels exceeding 10-fold and appropriately examined for etiology were included in the study. The markers for hepatothropic viruses and the others, autoimmune markers and hepatobiliary ultrasound were evaluated.

Results: In this study, 111 patients were included, 46 (41%) were female and 41.4% of the patients had AH-A, 17.1% had AH-B, 2.7% had AH-C and 6.3% were not found any cause. The mean age was 22.11±6.05 years in AH-A. The majority of AH-A cases were male with 65%. The mean age was 33.5±14.78 in AH-B. There was a statistically significant difference between ages of patients with AH-A and AH-B (p=0.004).

Conclusion: Since no specific treatment is available for acute viral hepatitis, preventive measures are more significant. The prevalence of AH-A and AH-B have declined in the recent years. It is clear that extensive vaccine policies and improved sanitation help eliminate these diseases. In order to ensure complete elimination of viral hepatitis, it is essential to give due importance to the vaccination in childhood as well as in adults.

Keywords: Acute hepatitis, virus, transaminase

ÖZ

Amaç: Hastanemize akut hepatit ile başvuran erişkin hastaların etiyolojik, epidemiyolojik ve laboratuvar özelliklerini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya alanin aminotransferaz (ALT) düzeyleri 10 katın üzerinde olan ve etiyolojisi uygun olarak incelenen hastalar dahil edildi. Hepatothropik virüsler ve diğerleri için belirteçler, otoimmün belirteçler ve hepatobiliyer ultrason değerlendirildi.

Bulgular: Çalışmaya 111 hasta dahil edildi, 46 (%41) kadın ve hastaların %41,4'ü AH-A, %17,1'i AH-B, %2,7'si AH-C ve %6,3'ü herhangi bir neden bulunmadı. AH-A'da ortalama yaş 22,11±6,05 yıldı. AH-A olgularının çoğu %65 ile erkekti. AH-B'de ortalama yaş 33,5±14,78 idi. AH-A ve AH-B'li hastaların yaşları arasında istatistiksel olarak anlamlı fark vardı (p=0,004).

Sonuç: Akut viral hepatit için spesifik bir tedavi mevcut olmadığından, önleyici tedbirler daha önemlidir. AH-A ve AH-B prevalansı son yıllarda azalmıştır. Kapsamlı aşı politikalarının ve iyileştirilmiş sanitasyonun bu hastalıkları ortadan kaldırmaya yardımcı olduğu açıktır. Viral hepatitin tamamen ortadan kaldırılmasını sağlamak için, aşılamaya yetişkinler kadar çocuklukta da gereken önemi vermek gerekir.

Anahtar Kelimeler: Akut hepatit, virüs, transaminaz

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Introduction

Acute hepatitis (AH) refers to necro-inflammation of liver which have many causes including viruses, drugs, alcohol, ischemia, autoimmune disorders and other causes. The most common causes of AH are hepatotropic viruses which have diverse types of transmission and epidemiologies. Hepatitis A virus (HAV), HBV, HCV, HDV and HEV are among most frequently observed in clinical practice (1) 852 patients with acute viral hepatitis from 17 centers were included in this study. Their sociodemographic characteristics, clinical courses, treatments, and laboratory findings were retrospectively analyzed. Results: The most commonly found microorganisms were the hepatitis B virus (55.2%. These are important health problem commonly seen both in our country and in the world. With increasing the administration of the vaccines worldwide, of the etiologies of AH, the prevalence of HAV and HBV has declined in the recent years (2) acute viral hepatitis most frequently is caused by infection with any of three distinct viruses: hepatitis A virus (HAV. Therefore, the epidemiology of AH has been changed. In this study, we aimed to evaluate the etiological, epidemiological and laboratory characteristics of adult patients admitted to our hospital with AH.

Materials and Methods

The patients who were followed up in the clinic of the Department of Infectious Diseases and Clinical Microbiology of Cerrahpaşa Medical Faculty between 2001 and 2019 were examined. Patients with alanine aminotransferase (ALT) levels exceeding 10-fold and appropriately examined for etiology were included in the study. The patients were retrospectively analyzed in terms of demographic data, etiology, age, gender, physical examination, laboratory findings, imaging methods and prognosis. Patients with missing diagnostic data were excluded from the study. The markers for hepatothropic viruses [anti-HAV immunoglobulin M (IgM), hepatitis B surface antigen, anti-HBc IgM, anti-HCV, anti-HDV, HCV-RNA] and the others [Epstein-Barr virus (EBV), Varicella zoster virus (VZV), Cytomegalovirus, etc], autoimmune markers and hepatobiliary ultrasound were evaluated.

Statistical Analysis

Data analysis was performed by using the SPSS 20.0 program. The laboratory values of patients were compared with univariate analysis. Subsequently, chi-square test and Mann-Whitney U test were used for categorical variables and continuous variables, respectively. A $p \leq 0.05$ was considered as statistically significant.

Results

In the study, 111 patients were included, 46 (41%) were female and 65 (59%) were male. Forty-six (41.4%) had AH-A, 19 (17.1%) had AH-B, 3 (2.7%) had AH-C and 7 (6.3%) were not found any cause. The mean age was 34.5±19.03 (minimum: 16, maximum: 89). In the first admissions to hospital, the mean aspartate aminotransferase (AST) was 1313.54±1158.46 U/L, the mean ALT was 1672.79±1132.42 U/L and the mean serum direct bilirubin was 4.70±3.76 mg/dL.

The ages ranged from 16 to 39 and the mean age was 22.11±6.05 years in AH-A. The majority of AH-A were male with 65%. The mean AST and ALT levels were 1586.70±1434.84 and 2096.04±1165.04 in AH-A respectively.

The ages ranged from 18 to 75 and the mean age was 33.5±14.78 in AH-B. The mean AST and ALT levels were 1474.42±796.27 and 2133.95±1103.90 in AH-B respectively. HDV coinfection did not occur in any case. All AH-B patients developed immunity except 2 patients. Seroconversion occurred in only one of the patients with acute flares of chronic hepatitis B. In acute flares of chronic hepatitis, only one patient developed immunity.

Five patients died from fulminant hepatitis including AH-B (1 patient), acute flares of chronic hepatitis B (1 patient) and unknown causes (3 patients). Toxic hepatitis was caused by ornidazole (1 case), cefazolin (1 case) and polypharmacy (1 case). Hepatic transaminases of all patients returned to normal limits after withdrawal of the drugs.

Hepatic tuberculosis was seen in a patient and the transaminases returned to normal ranges under anti-tuberculosis treatment.

The other etiologies of our patients were autoimmune hepatitis, leptospirosis, ischemic hepatitis, VZV, EBV, tuberculosis, reactive hepatitis and acute cholecystitis (Table 1).

Discussion

Acute viral hepatitis (AVH) is the most common liver disease in the world. Its prevalence varies according to socioeconomic and geographical characteristics of the countries. AH-A is frequently seen in childhood in developing countries (1) 852 patients with acute viral hepatitis from 17 centers were included in this study. Their sociodemographic characteristics, clinical courses, treatments, and laboratory findings were retrospectively analyzed. Results: The most commonly found microorganisms were the hepatitis B virus (55.2%. HBV and HAV are the first two common viruses in many adult AVH case studies. While some studies have reported type A predominance in AH (3,4), many studies have shown that

Table 1. The etiological distribution in patients presenting with acute hepatitis

Etiology of patients	No. of patients	Percent
Acute hepatitis A	46	41.4
Acute hepatitis B	19	17.1
Acute flares of chronic hepatitis	13	11.7
Acute cholecystitis	10	9.0
Unknown	7	6.3
Toxic hepatitis	3	2.7
Acute hepatitis C	3	2.7
Autoimmune hepatitis	2	1.8
Leptospirosis	2	1.8
Ischemic hepatitis	2	1.8
Varicella-zoster virus	1	0.9
Epstein barr virus	1	0.9
Tuberculosis	1	0.9
Reactive hepatitis	1	0.9
Total	111	-

type B hepatitis is more common (1,5,6) 852 patients with acute viral hepatitis from 17 centers were included in this study. Their sociodemographic characteristics, clinical courses, treatments, and laboratory findings were retrospectively analyzed. Results: The most commonly found microorganisms were the hepatitis B virus (55.2%. For example, in a study, HBV and HAV rates were seen 60.4% and 27.5% in AVH adult patients respectively (6). In our study, 41.4% of the patients were type A, followed by type B with 17.1%. On the other hand, it was observed that the AVH cases have markedly declined in the last ten years (Figure 1).

In a study, Eker et al. (7) found that the mean age for AH-A and AH-B was 21.5 and 33, respectively. In other studies, the mean age was found to be lower in patients with AH-A (8,9). In our study, the mean age for AH-A and AH-B cases are 22.1 and 33.5 respectively. There was a statistically significant difference between ages of patients with type A and with type B (p=0.004).

When the studies in our country examined in terms of gender, Çolpan et al. (9); 42.4% female, 57.5% male, Koruk et al. (8); 50% female and 50% male, Özkurt et al. (5); 42.7% female, 57.2% male were reported. In our study, 46 (41%) were female and 65 (59%) were male. While the incidence of AH-B did not differ between genders, the majority of AH-A cases were male.

In this study, AH-A and AH-B cases were mostly seen during autumn and winter months (Figure 2) and similar results were

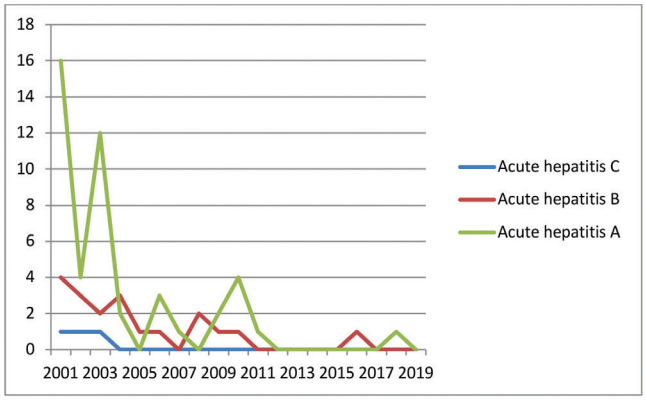

Figure 1. The change of the acute viral hepatitis by years

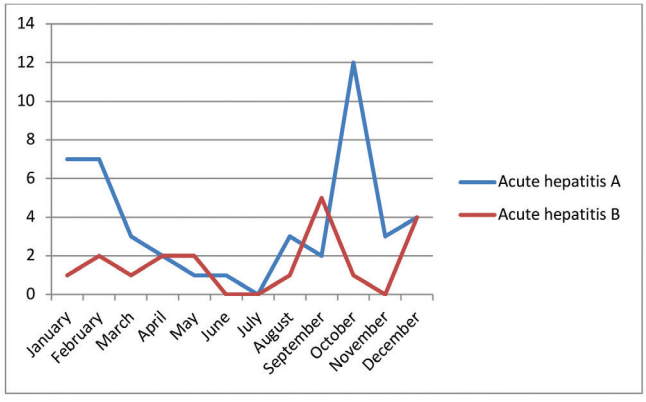

Figure 2. The number of cases of acute viral hepatitis by months

found in the previous studies (1,5) 852 patients with acute viral hepatitis from 17 centers were included in this study. Their sociodemographic characteristics, clinical courses, treatments, and laboratory findings were retrospectively analyzed. Results: The most commonly found microorganisms were the hepatitis B virus (55.2%).

In Turkey, the rate of positive hepatitis C antibody results is 1.12% among all groups (10). AH-C has generally subclinic and anicteric presentation and it is often difficult to differentiate acute infection from chronic infection with available tests. In our study, three (2.7%) AH-C cases were diagnosed with the presence of hepatitis symptoms, more than 10-fold increase in transaminases with anti-HCV and HCV-RNA positivity. Risk factors were not determined in any cases. The prevalence of AH-C was found to be high (2.7%), compared to other studies conducted in our country (1,5) 852 patients with acute viral hepatitis from 17 centers were included in this study. Their sociodemographic characteristics, clinical courses, treatments, and laboratory findings were retrospectively analyzed. Results: The most commonly found microorganisms were the hepatitis B virus (55.2%).

In the etiology of fulminant hepatitis, the most commonly found infectious agents are HAV in children and HBV in adults (11) gender, etiology, treatment modality, and outcomes. RESULTS A total of 308 patients were analyzed. Hepatitis A (20.9%). Fulminant hepatitis caused by HBV ranges from 0.1% to 0.4% (12). However; the rate was 4.5% (5/111) among all cases. Contrary to previous studies, in our patients, fulminant hepatitis more frequently developed due to unknown etiologies, not in HBV and HAV.

In another study conducted in our clinic between 1989-1991, majority of the patients (55.3%) were male and the rate of AH-A, AH-B and AH-C were 31.2%, 63.8% and 5% respectively (13). Three patients (2.8%) developed fulminant hepatitis and all of them died (13). Compared with our study, it was observed that the rate of AH-B and AH-C decreased over the years

Study Limitations

This study had some limitations including retrospective design, low number of cases and the lack of clinical symptom and signs. Prospective studies are required to better demonstrate these findings.

Conclusion

Since no specific treatment is available for AVH, preventive measures are more important to fight these diseases. In our study, though most of AVH were caused by HAV, followed HBV, the prevalence of AH-A and AH-B have declined in the recent years. It is clear that extensive vaccine policies and improved sanitation help eliminate these diseases. In order to ensure complete elimination of viral hepatitis, it is essential to give due importance to the vaccination in childhood as well as in adults.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Since our study was retrospective, informed consent was not used.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K., S.Y.K., Concept: Ö.F.T., Design: B.M., İ.İ.B., Data Collection or Processing: A.K., S.Y.K., Analysis or Interpretation: N.S., Ö.F.T., Literature Search: A.K., Writing: A.K., S.Y.K.

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Impact of COVID-19 Pandemic on Treatment, Follow-up and Behavioral Characteristics of Chronic Viral Hepatitis Patients

COVİD-19 Pandemisinin Kronik Viral Hepatit Hastalarının Tedavisi, Takibi ve Davranış Özellikleri Üzerindeki Etkisi

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ABSTRACT

Objectives: It has been not yet fully understood whether chronic liver diseases may be considered as risk factors for critical course of Coronavirus disease-2019 (COVID-19). Considering the importance of managing with COVID-19, we aimed to investigate the impact of the COVID-19 pandemic on treatment, follow-up and behavioral characteristics of chronic viral hepatitis (CVH) patients.

Materials and Methods: Patients followed-up in our clinic with diagnosis of chronic hepatitis B or C were screened retrospectively and a total 213 adults included in the study. We performed a telephone survey with patients.

Results: The mean age of participants was 49.9±13.4 years. Of the participants, 62% were male. Totally 75 (35.2%) patients disrupted their follow-up visits due to COVID-19 pandemic. The only risk factor for disruption in follow-up was found as anxiety. The vast majority of CVH patients paid attention to prevention measures for COVID-19.

Conclusion: We can say that CVH patients' awareness about COVID-19 and application of control measures were well enough. Continuity of treatment can be provided in patients with chronic illness during crisis period with informing patients in a way not to cause anxiety and efficient implementations in healthcare system.

Keywords: Anxiety, chronic viral hepatitis, COVID-19

ÖZ

Amaç: Kronik karaciğer hastalıklarının Koronavirüs hastalığı-2019'un (COVİD-19) kritik seyri için bir risk faktörü olup olmadığı henüz net olarak anlaşılamamıştır. COVİD-19 ile mücadelenin önemi göz önünde bulundurularak, COVİD-19 pandemisinin KVH hastalarının tedavi, takip ve davranış özellikleri üzerindeki etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Kronik hepatit B veya C tanıları ile kliniğimizde takip edilen hastalar retrospektif olarak taranmış ve toplam 213 erişkin hasta çalışmaya dahil edilmiştir. Hastalar ile telefon görüşmesi yapılarak anket gerçekleştirilmiştir.

Bulgular: Hastaların ortalama yaşı 49,9±13,4'tür. Katılımcıların %62'si erkektir. COVİD-19 pandemisi nedeni ile toplam 75 (%35,2) hasta takip vizitlerini aksatmıştır. Takibin aksatılmasındaki tek risk faktörü anksiyete bulunmuştur. KVH hastalarının büyük çoğunluğu COVİD-19'a yönelik korunma önlemlerine dikkat etmektedir.

Sonuç: KVH hastalarının COVİD-19 farkındalıklarının ve kontrol önlemlerine uyumlarının oldukça iyi olduğu görülmüştür. Kriz dönemlerinde anksiyeteye neden olmayacak şekilde hastaların bilgilendirilmesi ve sağlık bakım sisteminde etkili uygulamalar sayesinde kronik hastalığı olan kişilerin tedavilerinin devamlılığı sağlanabilir.

Anahtar Kelimeler: Anksiyete, kronik viral hepatit, COVİD-19

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Introduction

At the end of 2019, an outbreak of atypical pneumonia with an unknown etiology appeared in Wuhan, Hubei Province, China (1). The disease caused by novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has been officially named as the Coronavirus disease-2019 (COVID-19) by World Health Organization (WHO). The outbreak has been spread rapidly all over the world and became a major public health problem (2). WHO declared the COVID-19 outbreak as a pandemic on March 11, 2020 and Europe was declared as a new epicenter of outbreak on March 14, 2020 (3,4). The first COVID-19 case was reported in Turkey on March 11, 2020 (5). Numerous measures and policy have been implemented as a response to COVID-19 outbreak in our country (4).

Older patients and those with pre-existing comorbidities have been defined as risk groups for severe course of COVID-19 (6). A cohort study enrolling 1099 COVID-19 patients in China showed that 21(2.1%) patients had pre-existing chronic hepatitis B (7). Recently, a small case series consisted of three patients with confirmed COVID-19 and decompensated cirrhosis (one due to chronic hepatitis B) were published, they have call attention to that decompensated cirrhosis may be a risk factor for critical course of COVID-19 patients (8). It has been not yet fully understood whether chronic liver diseases may be considered as risk factors for COVID-19 infection (6). During COVID-19 pandemic, it is essential to understand how the people have been coping with such a major threat on health. (1). There are no published data regarding the impact of COVID-19 infection on patients with chronic viral hepatitis (CVH) in current reports. Our study aimed to investigate the impact of the COVID-19 pandemic on treatment, follow-up and behavioral characteristics of chronic hepatitis patients.

Materials and Methods

Participants

This study is a cross-sectional study conducted as a telephone questionnaire between 5 June 2020 and 8 June 2020. Adults older than 18 years who had been followed-up with the diagnosis of chronic hepatitis B or C in our clinic were screened retrospectively and had agreed to participate in this study were included. Any monetary rewards were not given participants for completing the survey.

The study protocol and procedures of informed consent were approved by Ankara City Hospital Ethical Committee (approval number: E1/724/2020, date: 04/06/2020). Participants were asked if they accept participating to the study at the beginning of the questionnaire and an informed consent was provided by answering a yes-no question.

Survey

The telephone survey consisted of four parts with 25 questions about demographics features, treatment and follow-up of CVH, anxiety and risk assessment of patients about COVID-19 and behavioral changes of participants after COVID-19 pandemic. Prior to starting to questionnaire, participants were informed that participation was voluntary and they could stop at any time.

The first part of survey included basic demographic variables including age, gender, education, occupation, and place of current residence, comorbidities and medications. The second part consisted of the questions regarding if there is a break on the follow-up and/or treatment of CVH due to COVID-19 pandemic. The third part involved how participants perceive their own risk category for COVID-19 infection. In this part, additional questions were also asked about whether they were admitted to hospital and/or whether any investigation was made on preliminary diagnosis of COVID-19. The last part included nine questions asked to determine change in behavioral characteristics of patients after COVID-19 pandemic. They were asked whether if they made any changes daily routine behaviors such as avoiding going to out, using the public transport, staying away from crowded areas, meeting with relatives and friends, preferring internet shopping instead of markets, nutritional habits.

Statistical Analysis

Statistical analysis was performed by using SPSS software version 18.0. The results of descriptive statistics were reported as mean ± standard deviation for variables with normal distribution and median (minimum-maximum) for variables without normal distribution. Categorical data were reported as frequencies and percentages. Chi-square test was used to assess if there was a significant association between categorical variables. Student's t-tests was used to determine differences between numerical values.

Results

A total 213 participants were included into the study (Table 1). The mean age of participants was 49.9±13.4 years, and 50.7% were over 50 years of age. Of the participants, 62% were male and 46.9% had higher level of education. Of the patients, 35.6% had at least one comorbidity and the most common comorbidities were hypertension and diabetes mellitus. Duration of CVH B and C were 12.6±6.08 and 10.7±4.75 years, respectively. One third of chronic hepatitis B patients received tenofovir disoproxil fumarate or entecavir treatment. All of the chronic hepatitis C patients had completed their antiviral treatment before COVID-19 pandemic.

While treatment interruption was seen in only three CVH patients, totally 75 (35.2%) patients disrupted their follow-up visits due to COVID-19 pandemic. The most common reason was the afraid of admission to hospital (-21.1%). In addition, 14 patients voluntarily disrupted their outpatient follow-up for reasons other than COVID-19. All causes of follow-up disruption were listed in Table 2. The investigated risk factors for follow-up disruption were showed in Table 3. The only statistically significant risk factor which negatively affect the follow-up in CVH patients was found as anxiety about being at higher risk for COVID-19 due to hepatitis (p=0.002).

Fifty-three (24.9%) CVH patients were anxious for being at higher risk for COVID-19 due to hepatitis disease. Causes of their anxiety were the thought of carrying a higher risk for acquiring of the infection or having more severe forms of COVID-19 infections due to hepatitis (14.6%), thought of being immunosuppressive due to hepatitis (13.6%) and fear of having more side effects of COVID-19 drugs during treatment due to hepatitis (2.3%). Baseline characteristics of patients with and without anxiety about COVID-

19 were similar according to age, education level, prescence of any comorbidity, type of hepatitis and duration of illness. The only statistically significant factor that caused anxiety was found as gender (p=0.010).

Only 3 (1.4%) patients applied to hospital with suspicion of COVID-19. All of them had a negative COVID-19 polymerase chain reaction test and normal thorax computerized tomography. Of the patients, 7 (3.3%) had a person in own family or close social environment who have been infected with COVID-19. None of the CVH patients were diagnosed with COVID-19.

Table 1. Characteristic features of patients with chronic viral hepatitis

Total participants, n	**213**
Age, years, (n, %)	
18-30	12 (5.6)
31-40	54 (25.4)
41-50	39 (18.3)
>50	108 (50.7)
Male, (n, %)	132 (62)
Education (n, %)	
Illiterate	11 (5.16)
Primary education	82 (38.5)
Secondary school	20 (9.4)
High school	49 (23)
University	51 (23.9)
Occupation (n, %)	
Working	143 (67.1)
Not working	70 (32.9)
Any comorbidity other than hepatitis, (n, %)	78 (35.6)
Hypertension	40 (18.8)
Diabetes mellitus	23 (10.8)
Chronic pulmonary disease	6 (2.8)
Coronary artery disease	6 (2.8)
Solid organ malignancy	3 (1.4)
Rheumatologic disease	3 (1.4)
Hematological malignancy	1 (0.5)
Solid organ transplantation	1 (0.5)
Any medication, (n, %)	109 (51.2)
Chronic viral hepatitis B (n, %)	179 (84)
Duration of disease, years-mean (± standard deviation)	12.6 (6.08)
Family history (n, %)	89 (49.7)
Any antiviral treatment (n, %)	57 (31.8)
Treatment option (n, %)	
Tenofovir disoproxil fumarate	40 (22.3)
Entecavir	17 (9.5)
Chronic hepatitis C (n, %)	34 (16)
Duration of disease, years-mean (± standard deviation)	10.7 (4.75)
Ongoing antiviral treatment (n, %)	0 (0)

We examined the behavioral characteristics of hepatitis patients after COVID-19 pandemic (Table 4). All of the CVH patients except one have increased hand washing, using disinfectants and wearing face masks rates. A significant number of patients expressed themselves to pay attention to avoiding form crowded area, meetings and using public transport.

Discussion

The COVID-19 pandemic may cause additional psychological distress in the population (9). We learned from the past pandemics that approximately 35% of individuals experienced depressive or anxiety symptoms one month after SARS period (10). In our study, 24.9% of hepatitis patients were anxious for being at higher risk for COVID-19 due to hepatitis disease. Also these people who were anxious about COVID-19 have discontinued their further follow-up. According to The European Association for The Study of the Liver and The European Society of Clinical Microbiology and Infectious Disease, CHV doesn't appear to increase the risk of a critical course of COVID-19 (6). Higher level of knowledge should be provided, but this awareness should not lead to anxiety, depression or panic. An increasing amount of information and concerns are impacting on global mental health, so people may be exposed to misleading information by some social media platforms. Since misinformation sources may cause incorrect perception, discourses that may cause anxiety should be avoided in critical pandemic periods. Similar situation was reported in Italy in inflammatory bowel disease patients. They were severely worried about COVID-19 infection and its impact on their medication, disease prognosis. Therefore, Italy adopted several strategies for these special group of patients in order to maintain the quality standard of care (11). Higher pleasure from the health information received is associated with lower psychological impact of the epidemic, the content of information provided should be based on evidence to prevent an adverse, stressful and anxious reactions (12).

COVID-19 pandemic poses an extremely challenge to healthcare systems in affected countries (6). As well as the international organizations, positions of local authorities, health and other ministries, disease control centers, universities and research centers have critical importance in different levels to control the

Table 2. Discontinuance of follow-up in hepatitis B and C patients (n=213)

Discontinuance of follow-up, n (%)	**89 (41.8)**
Associated with COVID-19	75 (35.2)
Non-associated with COVID-19	14 (6.6)
Causes of follow-up disruption, n (%)	
"I am afraid of admission to hospital"	45 (21.1)
"I didn't admit to hospital because I couldn't reach my doctor"	19 (8.9)
"I thought that it would not be a problem to disrupt my follow-up"	10 (4.7)
"Because of the curfew"	5 (2.3)
"I kept in touch with my doctor who postponed my appointment"	4 (1.9)

COVİD-19: Coronavirus-2019

Table 3. The risk factors for discontinuance of follow-up (n=213)			
Patients characteristics	**Patients with disruption in follow-up (n=75)**	**Patients without disruption in follow-up (n=138)**	**p**
Sex			
Female	35 (46.7)	46 (33.3)	0.056
Male	40 (53.3)	92 (66.7)	
Age group			
18-30	6 (8)	6 (4.3)	0.056
31-40	25 (33.3)	29 (21)	
41-50	15 (20)	24 (17.4)	
>50	29 (38.7)	79 (57.2)	
Education level			
Illiterate	5 (6.7)	6 (4.3)	0.684
Primary education	27 (36)	55 (39.9)	
Secondary school	5 (6.7)	15 (10.9)	
High school	20 (26.7)	29 (21)	
University	18 (24)	33 (23.9)	
Presence of any comorbidity	27 (36)	51 (37)	0.890
Type of hepatitis disease			
Hepatitis B patients	68 (90.7)	111 (80.4)	0.051
Hepatitis C patients	7 (9.3)	27 (19.6)	
Duration of illness, years, median (minimum-maximum)	10 (4-30)	10 (1-40)	0.602
Anxiety of being at higher risk for COVID-19	28 (37.3)	25 (18.1)	0.002
COVID-19: Coronavirus-2019			

Table 4. Change of behavioral characteristics of hepatitis patients after COVID-19 pandemic (n=213)	
Behavioral characteristic	**n (%)**
Increase in hand washing, usage of disinfectants and wearing a face mask	212 (99.5)
Avoiding going to out unless it is necessary	209 (98.1)
Staying away from crowded areas	205 (96.2)
Avoiding from using the public transport	193 (90.5)
Staying away from meeting with relatives and friends	189 (88.7)
Not preferring to shop from the markets	91 (42.7)
Preferring to shop on the internet more frequently	33 (15.5)
Doing change in nutritional habits	31 (14.6)
Using herbal supplements	12 (5.6)
COVID-19: Coronavirus-2019	

pandemic (13). Turkey implemented numerous control measures immediately at the beginning of COVID-19 pandemic for managing with COVID-19 infection (13). One of these control measures was that people who used drugs regularly for chronic illnesses, can be provided the medications without any prescriptions or medical reports (14). They could obtain the medications directly from the pharmacy based on last medical reports (14). In this way, risk groups movement and admission to hospitals due to nonemergency issues were restricted. Because healthcare settings are one of the most important areas for prevention and control of viral transmission (6).

In our study, only three patients had treatment disruption by means of these efficient implementations in healthcare system. Since the face-to face contact can increase dissemination of the virus, different technical solutions can be found to enable remote physician-patient interactions, for example using telemedicine, visits by phone, sending follow-up prescription by mail (6). Use of telemedicine has increased dramatically last years (15). Routine follow-up of chronic disease, management and information of mild patients disease, rapidly triage of patient to the the related clinics can be provided by telehealth (15). Pandemics cause emerging of new unique challenges to health care delivery (16). We should accept that pandemic is a part of our lives and similar or new pandemis may appear at any time in the future. To be prepared for pandemics, it is necessary to work for rapidly implementation of telemedicine into the healthcare system.

In our study, only female gender was found as a risk factor for anxiety in hepatitis patients during COVID-19 pandemic (p=0.010**)**. Similar result was reported by Qiu et al. (17) in a nationwide survey about psychological distress among Chinese people during COVID-19 outbreak. They founded that female participants had significantly higher psychological distress than males (p<0.001) (17). In another study, female gender was found as a risk factor for a higher levels of stress, anxiety, and depression in COVID-19

pandemic (p<0.05) (12). These findings supported the previous epidemiological studies showing gender' effect on prevalence of depression (18). Socioeconomic or reporting bias can lead to emerging the gender differences in anxiety disorder, however, several studies suggest that fluctuating levels of estradiol and progesterone play an important role in these differences (19). For this reason, sociodemographic information is very important for healthcare authorities to identify for high risk groups and to act early intervention during pandemic (12).

The vast majority of CVH patients paid attention to prevention measures for COVID-19 infection like hand washing, wearing mask, not going crowded area etc. These results were in agreement with the report of Zhong et al. (20). These high proportion of consistence to prevention measures could be related with participants' good knowledge about COVID-19 infection transmission routes (20). CVH patients were less impressed by outbreak, since these people isolated oneself due to chronic illness. Likewise, patients having different types of immunodeficiency switched to remote assistance program in Italy (21). It was showed that the remote assistance program didn't have negative impact on health-related quality of life of these patients (21).

Study Limitations

A number of limitations of this study should be noted. First, the size of sample was small. Second, the study included only of CVH patients who were followed-up in single center. Further studies with large multi-center samples are needed.

Conclusion

This study intended to examine the impact of the COVID-19 pandemic on treatment, follow-up and behavioral characteristics of chronic hepatitis patients. Despite we investigated the impact of COVID-19 infection in a limited number of hepatitis patients, we can say that CVH patients' awareness about COVID-19 and application of control measures were well enough. We detected anxiety as the only factor causing follow-up interruption. Informing patients in a way not to cause anxiety is very important to prevent any disruption in the follow up- and treatment of the primary disease. By means of efficient implementations in healthcare system, continuity of treatment can be provided in patients with chronic illness during crisis period like COVID-19 pandemic.

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Ethics

Ethics Committee Approval: The study protocol and procedures of informed consent were approved by Ankara City Hospital Ethical Committee (approval number: E1/724/2020, date: 04/06/2020).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.G., B.K., Design: R.G., B.K., E.M.S., Data Collection or Processing: B.K., E.M.S., Analysis or Interpretation: R.G., B.K., E.M.S., Literature Search: R.G., B.K., E.M.S., Writing: R.G., B.K., E.M.S.

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Research Article

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Evaluation of Hepatitis A Seroprevalance and Epidemiologic Data of Patients Applying to A Medical Faculty Hospital

Bir Tıp Fakültesi Hastanesine Başvuran Hastalarda Hepatit A Seroprevalansı ve Epidemiyolojik Verilerin Değerlendirilmesi

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ABSTRACT

Objectives: Our study aims to determine seroprevalence of hepatitis A virus (HAV) in our region and to determine the prevalence change over years and to evaluate the effects of various factors on prevalence.

Materials and Methods: In our study, HAV diagnostic tests, which were studied by the ELISA method in the microbiology laboratory of our hospital for 10 years, were evaluated retrospectively from the laboratory information system. In the diagnosis of acute hepatitis, and anti-HAV immunoglobulin M (IgM) antibody was investigated in the blood, and anti-HAV IgG antibody was investigated for immunity to hepatitis A.

Results: Seropositivity of anti-HAV IgG was found to be 75.3%, In comparison, while anti-HAV IgM positivity was found to be 2.7%. While anti-HAV IgG and anti-HAV IgM positivity were found to be higher in individuals living outside the city center compared to individuals living in the city center, no significant difference was found between the genders.

Conclusion: Virus transmission has decreased in our region in recent years, and the encounter with the disease has shifted to advanced ages. This change increases the number of individuals susceptible to symptomatic infection. Therefore, hygiene and sanitation conditions should be monitored carefully, infrastructure should work adequately, and vaccination policies should be implemented when necessary.

Keywords: HAV seroprevalence, anti-HAV IgG, anti-HAV IgM

ÖZ

Amaç: Bu çalışmada yöremizde hepatit A virüs (HAV) seroprevalansının belirlenmesi ve yıllar içinde prevalansta oluşan değişimin tespit edilerek, çeşitli faktörlerin prevalans üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamızda, 10 yıllık süreçte hastanemiz mikrobiyoloji laboratuvarında ELİSA yöntemiyle çalışılan HAV tanı testleri laboratuvar bilgi sisteminden geriye dönük olarak değerlendirilmiştir. Akut hepatit tanısında kanda anti-HAV immünoglobulin M (IgM) antikor varlığı incelenirken, hepatit A'ya karşı geliştirilen bağışıklık için ise kanda anti-HAV IgG antikor varlığı araştırılmıştır.

Bulgular: Çalışmada anti-HAV IgG seropozitifliği %75,3 oranında bulunurken, anti-HAV IgM pozitifliği %2,7 oranında bulunmuştur. Anti-HAV IgG ve anti-HAV IgM pozitiflikleri şehir merkezi dışında yaşayan bireylerde, şehir merkezinde yaşayan bireylere göre yüksek bulunurken, cinsiyetler arasında anlamlı bir farklılık saptanmamıştır.

Sonuç: Yöremizde son yıllarda virüs bulaşı azalmış ve hastalıkla karşılaşma ileri yaşlara kaymıştır. Bu değişim semptomatik enfeksiyona yatkın duyarlı birey sayısını artırmaktadır. Bu nedenle hijyen ve sanitasyon koşulları dikkatli takip edilmeli, altyapı düzgün çalışmalı, gerekli durumlarda aşılama politikaları uygulanmalıdır.

Anahtar Kelimeler: HAV seroprevalans, anti-HAV IgG, anti-HAV IgM

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Introduction

Hepatitis is an inflammatory disease of the liver characterized by hepatocellular injury (1). The primary etiology of acute hepatitis is viral. Despite all advances, viral hepatitis is still a significant cause of mortality and morbidity worldwide (2). Hepatitis A Virus (HAV) is a non-enveloped-RNA virus in the Picornaviridae family and is the most common cause of acute hepatitis worldwide. The disease has different forms, ranging from asymptomatic hepatitis to fulminant hepatitis, with no chronicity (3).

Fecal excretion in HAV infections is the primary source of the virus. HAV, primarily transmitted by the fecal-oral route, has different pathways (4). Accurate determination of the prevalence of hepatitis A infections becomes difficult due to the excess of asymptomatic patients and inadequate hospital discharge reports (5). In the definite and specific diagnosis of acute hepatitis A, the detection of immunoglobulin M (IgM) antibodies developing against HAV is used. Anti-HAV IgG, which has protective and virus-neutralizing properties, is positive within a few weeks of infection and may remain positive for decades as an indicator of immunity (6).

The epidemiology of HAV varies greatly geographically. Hygiene conditions, access to clean water resources, and other socioeconomic conditions are the leading causes of geographical differences in the prevalence of HAV infection. Even if the improvement in these primary conditions reduces the incidence of HAV, especially in developed countries, infection in developing countries still has a high incidence. Routine vaccination programs in countries also affect the epidemiology of HAV (7). In general, improvement in hygiene and sanitation conditions leads to a decrease in the number of cases, while seropositivity seems to shift to advanced ages (8).

In terms of HAV infection, our country is in the middle endemicity region and our country may have different rates in terms of seroprevalence in its regions and with other countries (6). To determine the preventive measures related to hepatitis A, a disease affecting large masses of the population, causing mortality as well as high morbidity, it is very essential to determine the prevalence of the disease in that society and monitor the prevalence change over the years (9). Our study aimed to determine seroprevalence of HAV in Sivas province, change in prevalence over the years, and evaluate the effects of factors on prevalence.

Materials and Methods

Our study was conducted at Sivas Cumhuriyet University Faculty of Medicine Hospital, a tertiary level education and research hospital with 1150 beds and 1544 polyclinics/day-patient capacity. In our study, the serology test results obtained from the samples sent from various departments to our laboratory between 2008-2017 were investigated retrospectively from the laboratory information system.

In these serology tests, the presence of anti-HAV IgM antibody in the blood was investigated in the diagnosis of acute hepatitis and the presence of anti-HAV IgG antibody in the blood was investigated for immunity to hepatitis A.

In order to detect anti-HAV antibodies in the serum within 2 hours at the latest, 3-5 mL blood samples taken from the EDTA tubes were separated into their sera by centrifugation in the laboratory. They were qualitatively analyzed according to the manufacturer's procedure using Chemiluminescent Microparticle Enzyme Immunological Test method with Architect i2000SR (Abbott Diagnostics, Illinois, USA) and Architect test kits (Abbott, Wiesbaden, Germany). Lipemic and hemolysed sera were not analyzed. Repeated results of the same patients for serology test parameters (IgG and IgM) were not used in the study.

Statistical Analysis

The data obtained in our study were entered into SPSS (version 22.0), and the data was evaluated by using the chi-square test in 2x2 schemes. The Fisher's exact chi-square test was used when the hypothesis was not fulfilled, and the chi-square test was used in the multi-chamber schemes. The level of error was taken as $p<0.05$.

Results

In this study, 21,578 anti-HAV test results of patients who applied to the outpatient clinics and clinics of Sivas Cumhuriyet University Application and Research Hospital between 2008 and 2017. The 21,578 anti-HAV test results from blood samples sent to the microbiology laboratory on suspicion of hepatitis and tested with the ELISA method were retrospectively examined in the patient records of the laboratory. The anti-HAV IgG test parameter was evaluated in 10,550 of these test results, and the anti-HAV IgM parameter was evaluated in terms of positivity and negativity in the remaining 11,028 test results. Anti-HAV IgG positivity was found to be 75.3% in all patients, and anti HAV IgM positivity was 2.7% in all patients (Table 1).

When HAV IgG and HAV IgM test results were evaluated in terms of gender, there was no statistically significant difference between the genders in terms of seropositivity ($p>0.05$) (Table 2).

In our study, relatively low positivity rates were observed between 0-10 years, 11-20 years, and 21-30 years and seropositivity rates increased in older age groups. As a result, there is a difference between 0-10 years, 11-20 years, 21-30 years, and other age groups in terms of positivity rate. When anti-HAV IgG and anti HAV IgM positivity were evaluated according to age groups, the differences were significant ($p<0.05$) (Table 3).

When anti-HAV IgG positivity was compared by the years, the difference was significant ($p<0.005$). The lowest positivity rate was found in 2009 and 2010, and as the year increased, the positivity rate increased. The highest positive rate was observed in 2016 and 2017. When the anti-HAV IgM positivity rates were compared

Table 1. Distribution of HAV diagnostic tests between 2008-2017

Test parameter		Result		Total
		Positive	Negative	
Anti-HAV IgG	n	7,940	2,610	10,550
	%	75.3	24.7	100
Anti-HAV IgM	n	301	10,727	11,028
	%	2.7	97.3	100
Total	n	-	-	21,578
	%	-	-	100

HAV: Hepatitis A virüs, Ig: Immunoglobulin

by years, the difference was also significant (p<0.05). The highest positivity rates were observed in 2008, 2009, and 2011, while the positivity rate gradually decreased in the following years (Table 4).

When the anti-HAV IgG positivity of 9,212 patients, whose address information can be reached, were examined by location and when the anti-HAV IgM positivity of 9,665 patients was examined, it was seen that the positivity rates of those living outside the city center were higher than those living in the city center (Table 5).

Discussion

HAV infection is a common type of infectious hepatitis that is common all over the world, especially in developing countries. HAV, which is spread via fecal-oral transmission, infects millions of people worldwide every year. Crowded living conditions, low income, and education, living in rural areas and slums, access to clean water are independent risk factors. Despite its low mortality, HAV infection is still a substantial public health problem as it causes outbreaks and loss of labor (8,10).

Seroepidemiological data are of great importance to prevent common hepatitis A infections by developing effective prevention and vaccination strategies (11). Hepatitis A seroprevalence varies according to age groups, country, region, and hygiene/sanitation conditions. Therefore, it is more critical to monitor age-specific prevalence and change in prevalence over the years rather than the average prevalence (12).

Although Turkey may be considered as one of the moderate endemicity countries in terms of HAV infection, endemicity in their geographic area, age, and socioeconomic status may vary according to (10). In our study, the anti-HAV IgG positivity rate was

Table 2. Comparison of anti-HAV IgM and anti-HAV IgG test positivity between 2008 and 2017 by gender (%)

Test parameter	Gender		Total
	Female	Male	
Anti-HAV IgG	75.9	74.6	75.3
Anti-HAV IgM	2.6	2.8	2.7

HAV: Hepatitis A virüs, Ig: Immunoglobulin

Table 3. Comparison of anti-HAV IgG and anti-HAV IgM test positivity between 2008-2017 by age groups (%) (IgG n=10550, IgM n=11028)

Age group		Test parameter	
		IgG	IgM
0-10 age	n	668	108
	%	36.5	4.9
11-20 age	n	578	93
	%	46.5	6.8
21-30 age	n	1,269	50
	%	64.7	3.1
31-40 age	n	1,109	13
	%	94.4	1.1
41-50 age	n	1,135	9
	%	99	0.8
51-60 age	n	1,205	9
	%	99.8	0.7
61-70 age	n	1,046	7
	%	99.2	0.6
71-80 age	n	708	11
	%	99.9	1.3
81-90 age	n	213	1
	%	98.6	0.3
91-100 age	n	9	0
	%	100	0
Total	n	7,940	301
	%	75.3	2.7

HAV: Hepatitis A virüs, Ig: Immunoglobulin

Table 4. Comparison of anti-HAV IgG and anti-HAV IgM test positivity between years 2008-2017 (%) (IgG n=10550, IgM n=11028)

Year		Test parameter	
		IgG	IgM
2008	n	896	90
	%	73.1	9.7
2009	n	867	55
	%	70.8	4.5
2010	n	809	36
	%	69.8	3.1
2011	n	840	72
	%	74.1	6.2
2012	n	594	21
	%	78.6	3
2013	n	384	9
	%	72.7	1.5
2014	n	504	2
	%	74.7	0.2
2015	n	550	3
	%	77	0.3
2016	n	714	4
	%	78.5	0.3
2017	n	1,782	9
	%	80.2	0.4
Total	n	7,940	301
	%	75.3	2.7

HAV: Hepatitis A virüs, Ig: Immunoglobulin

Table 5. Comparison of anti-HAV IgG and anti-HAV IgM test positivity between 2008-2017 by location

Region	IgG Total patient/positive (%)		IgM Total patient/positive (%)	
Center	5,981/4,389	73.4	5,992/144	2.4
Rural	3,231/2,577	79.8	3,673/140	3.8

HAV: Hepatitis A virüs, Ig: Immunoglobulin

found to be 75.3%, and anti-HAV IgM positivity was 2.7% for all patients undergoing the HAV IgG test between 2008-2017. Our results are consistent with other studies in the literature. Several studies in the Central Anatolia region have reported anti-HAV IgG antibodies between 77% and 81% (13,14). Seropositivity was found to be 69.7% in Afyonkarahisar and 78.8% in Çanakkale (15,16). In a study conducted in Rize from the Black Sea region, seropositivity was 75% (17). In a study conducted in Batman, southeast of the country, IgG antibody positivity was 93.9% (18). In a retrospective screening performed in Malatya, anti-HAV IgG seropositivity was found to be 74.4% (19). In Hatay, in the south of the country, seropositivity for the IgG antibody was 81.1% in 2007 (20). In some studies, it has been reported that the rate of seropositivity tends to decrease in our country (13). There was a significant decrease in the seroprevalence of HAV in Adana between 1998 and 2009 (21). In the general population study conducted by Poyraz et al. (22), in our province, anti-HAV IgG seropositivity was found to be 91% for all patients. When the study of Poyraz et al. (22), in our city was compared with our study, the significant decrease in anti-HAV IgG seropositivity appears to be an indicator of decreased virus circulation due to improvement in hygiene and sanitation conditions and socioeconomic conditions. In a retrospective study in Malatya, anti-HAV IgM positivity was reported to be 1.3% (19). In a study conducted in Konya, anti-HAV IgM positivity was 2.89%, whereas, in a study involving children and adults in Kırşehir, anti-HAV IgM positivity was 0.5% (14,23). Similar rates have been reported in other studies reported from our country (17,24,25). Rates ranging between 0.3 and up to 40% have been reported for anti-HAV IgM positivity from different geographic regions of the world (26,27,28,29).

When anti-HAV IgG seropositivity was compared during the 10 years, the lowest rate was found in 2009 and 2010, and the highest rate was seen in 2016 and 2017. In our study, we obtained a low seropositivity rate in 2009 and 2010. We think that this low rate will be due to two reasons: the high rate of asymptomatic hepatitis A infection especially before the age of 6 and the inability to detect outbreaks from the asymptomatic transmission chain created by children who are prone to infection in crowded places such as nursery, kindergarten, and school. It was determined that seropositivity increased continuously in 2017 and reached its highest rate in 2017. This continuous rising in seropositivity is thought to be due to increase in individuals applying hospital because of symptomatic disease, the contribution of viral contact to adolescents and young adults from the early childhood due to the improvement in hygiene and sanitation conditions, and the contribution of hepatitis A vaccine, which has been added to routine vaccine program as of 2012.

In our study, when the anti-HAV IgM positivity was compared by years, the difference was found to be significant ($p<0.05$). While the highest positivity was observed in 2008, 2009, and 2011 and the positivity rate decreased as the years increased, anti-HAV IgM positivity decreased sharply as of 2012 and reached its lowest rate for 2014. This situation is thought to be related to the increase in the number of seropositive children due to inclusion of hepatitis A vaccine in the routine vaccination program as of September 2012, improvement in access to clean water and sanitation, and decrease in the number of family members as a result of improvement in socioeconomic conditions over the years. Studies in Israel and the United States report that vaccination of young children significantly reduces the incidence of HAV in all age groups (30,31).

In our study, when we examined the distribution of anti-HAV IgG seroprevalence according to age groups, 36.5% to 100% were determined according to age groups within the range between 0 and 100 years of age (Table 3). In a study conducted in Diyarbakır, anti-HAV IgG seropositivity was found to be higher compared to our study results in terms of age groups (25). In a previous study conducted in Ankara, seropositivity was found 33.9% between 0-19 years of age and over 90% in all age groups over 30 years of age (13). In a study conducted in İstanbul, it was determined that while seropositivity in the 0-10 age group was 21%, it was 19% in the 11-20 age group. It was also reported in the same study that seropositivity was balanced in both groups in the 21-30 age group (50%) and it reached up to 81% after 30 years of age (32). Seropositivity, which was 55% in the 20-29 age group in İzmir, was reported to be over 90% after 40 years of age (33). In a previous study was conducted in the Çanakkale province, HAV seropositivity was found to be 61.4% for 17-21 years, while 96.3% over 52 years (16). In our study, while the lowest seropositivity was seen in 0-10, 11-20, 21-30 age groups, respectively, it increased to more than 90% in the 31-40 and later age groups and increased to 100%. In the previous study in our province, seropositivity was above 90% at age 11 and after (22). While in our study, seropositivity was above 90% at age 31 and after. This difference indicates that the initial age of contracting HAV has shifted to older ages due to the improvement in the conditions of hygiene and sanitation, and socioeconomic level.

When the relationship between age groups and anti-HAV IgM positivity was examined in our study, the highest positivity rate was seen in 0-10, 11-20, 21-30 age groups in terms of age groups. At age 31 and over, the positivity rate decreases with age ($p<0.05$). In our study, although the rate of acute HAV decreased gradually in the age groups, the high positivity rate in the 10-20 age group can be attributed to the contribution of viral contact to adolescents and young adults from early childhood due to the improvement in hygiene and sanitation conditions. Also, the hepatitis A vaccine given since 2012 is thought to have caused a lower anti-HAV IgM positivity rate in the 0-10 age group compared to the 11-20 age group.

In the previous study conducted in our city, anti-HAV IgM positivity rates for the age groups 3-10 and 11-20 years were found to be 7.2% and 0.8%, respectively, and 0% for subsequent age groups (22). When we compare this study with our study, it is seen that IgM positivity was found to be high in the 0-10 age group in the previous study, whereas in our study, IgM positivity reached the highest rate in the 11-20 age group. This suggests that the age of contracting HAV has shifted to advanced ages due to the reasons mentioned earlier. This change may lead to an increase in the number of adult individuals susceptible to symptomatic infection in the future.

In our study, no significant difference was found between the genders similar to the literature ($p>0.05$). Worldwide prevalence studies show that anti-HAV IgG and IgM seropositivity are similar between genders (26,29,34,35). In the study conducted by Poyraz et al. (22), in our province, anti-HAV IgG seropositivity was found

to be 89.1% and 92.7%, and anti-HAV IgM positivity was 1% and 1.4% in males and females, respectively. In a study conducted in Iğdır with patients aged 0-18 years, the rate of acute hepatitis A was 19.6% in females and 17.0% in males, and this difference was not significant (36). Other studies from our country have also shown that anti-HAV IgG and IgM seropositivity do not show a significant difference between genders (16,23,25,37).

Anti-HAV IgG and IgM seropositivity were higher in people living in rural areas for all age groups in our study ($p<0.05$). In a study covering the years between 2011 and 2014 in Konya, anti-HAV IgG seropositivity was found to be significantly higher in rural patients compared to urban patients, and living in rural areas was identified as an independent risk factor for anti-HAV IgG positivity (38). In another study conducted in Konya, anti-HAV IgM positivity was found to be significantly higher in the periphery districts compared to the central districts. In this study, anti-HAV IgM positivity was found to be the lowest in Meram district with high socioeconomic status (14). In the studies reported from our country and other parts of the world, seropositivity was found to be lower in individuals living in city centers (39,40). Seropositivity was higher in individuals living in rural areas than in individuals living in the city center. It is thought to be related to poor sanitation conditions, lack of sewage system low rate of chlorinated drinking water consumption, and to some socioeconomic conditions such as crowded families in rural areas.

Conclusion

In the last 20 years, improvement in socioeconomic and hygiene-sanitation conditions in our country and the world has led to changes in the epidemiological profile and pattern of hepatitis A. As is known, even in regions of a country, differences in the conditions as mentioned above create variations in prevalence. Therefore, epidemiological studies on the prevalence of HAV in any area will provide guide the prevention of outbreaks in that area, and the development of new prevention and vaccination strategies.

Ethics

Ethics Committee Approval: Ethics Committee approval was obtained from Sivas Cumhuriyet University Ethics Committee (approval number: 01/10, date: 01.01.2018).

Informed Consent: It was conducted in accordance with the Ethics Committee and approval procedures.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: C.Ç., M.S., Design: C.Ç., M.S., Data Collection or Processing: C.Ç., M.S., A.H.T.K, M.H., Analysis or Interpretation: C.Ç., M.S., A.H.T.K, M.H., Literature Search: C.Ç., M.S., Writing: C.Ç., M.S., A.H.T.K, M.H.

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